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JOURNAL OF THE FISHERIES RESEARCH BOARD OF CANADA

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Thecate Hydroids from the Shelf Waters of Northern Canada

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CALDER, D. R. 1970. Thecate hydroids from the shelf waters of northern Canada. J. Fish. Res. Bd. Canada 27: 1501–1547.

Based largely on collections from the *Calanus–Salvelinus* expeditions, 54 species of thecate hydroids were identified from the shelf waters of northern Canada between northeastern Newfoundland and the Alaska–Yukon border. Common species included *Halecium muricatum, Calycella syringa, Campanularia integra, C. speciosa, C. volubilis, Gonothyraea loveni, Filellum serpens, Lafoea gracillima, Sertularella polyzonias, S. tricuspidata, Sertularia schmidti,* and *S. similis. Halecium groenlandicum, H. scutum, Cuspidella procumbens, Calycella gracilis,* and *Sertularia schmidti* are new records for North America; *Ptychogena lactea* is previously known from this continent only as the medusa. Twenty-two species are reported in northern Canada for the first time, bringing to 71 the number of thecate species recorded from the region. Nearly half of the 71 species recorded are circumpolar in distribution, and over two-thirds transgress both arctic and subarctic zones.

Most samples had a paucity of hydroids, particularly those from the high arctic. Collection records indicate that the most favourable regions for hydroids in northern Canada are the Strait of Belle Isle, eastern Ungava Bay, eastern Hudson Strait, northern and southeastern Hudson Bay, Foxe Channel, and northern Foxe Basin.

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INTRODUCTION

THE HYDROID FAUNA OF NORTHERN CANADA is poorly known compared with that of other arctic regions, particularly Greenland and northern USSR. Of the relatively few studies published on Canadian Arctic hydroids, most have been based on small collections, usually from a restricted area. Information is preliminary at best on the species of the western arctic or even such regions as Ungava Bay and Foxe Basin. The hydroids of Labrador have received the most attention, with records by Packard (1863, 1867, 1891), Hincks (1868), Kirchenpauer (1884), Whiteaves (1901), Kramp (1932b), and Fraser (1944). Verrill (1879) identified three species from Cumberland Sound collected by the Howgate Polar Expedition of 1877–78. Broch (1907) discussed several species collected near Ellesmere Island during the Second Norwegian Arctic Expedition in the *Fram*. Fraser (1922) listed 25 hydroid species, most of which were collected in Hudson Bay and Hudson Strait during the Canadian Arctic Expedition of 1913–18. Further data on the hydroids of Hudson Bay

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and Hudson Strait were given by Fraser (1931). Kramp (1932b) studied the hydroids taken by the Godthaab Expedition to west Greenland, which occupied a number of stations in Canadian waters from Labrador to Ellesmere Island. Fraser (1933) listed two species from southeastern Foxe Basin, and later (Fraser, 1939) reported five species from the western Canadian Arctic. Fraser (1944) cited most of the previous literature on hydroids from the eastern arctic and subarctic of Canada and included some new records.

The oceanography and marine biology of Canada's arctic and subarctic shelf waters have been under study annually since 1947 by personnel of the Fisheries Research Board of Canada. Using M.V. *Calanus* and M.V. *Salvelinus*, stations have been occupied from the Strait of Belle Isle in eastern Canada to the Alaska border in the northwest. Substantial collections of marine animals have been made over a two-decade interval, the hydroids and bryozoans of which, together in one collection, have been deposited in the Museum of Natural Sciences, National Museums of Canada. The bryozoans of this collection were examined by Powell (1968); an account of the thecate hydroids is the purpose of the present report.

Thecates were found in material from 84 of the stations occupied by M.V. *Calanus* and M.V. *Salvelinus* (Fig. 1, Table 1). Including some previously unreported hydroids from arctic Canada in the collection of the Museum of Natural Sciences, a total of 54 species of thecates were identified from the shelf waters of northern Canada and are discussed here. Combining the species reported in previous papers with the present records, 71 species of thecate hydroids have been identified from the study area. This compares with Kramp's (1943) records of 69 species from west Greenland, 37 species from east Greenland, 80 species from Iceland, and 51 species from Spitzbergen. About 87 thecates were listed by Naumov (1960) for the northern seas of the USSR and 101 species were reported from the entire Alaska coast by Fraser (1937).

The synonymy listed for each species in this paper includes the literature for the immediate study area only, namely the waters extending northward from the coastline between the Strait of Belle Isle and the Alaska-Yukon border. A paucity of material from northern Newfoundland precluded a detailed treatment of the hydroids of that region. The distribution given for each species discussed refers to their presence in cold waters of the northern hemisphere only. Records from the northern North Atlantic were derived mainly from Kramp (1943), those from the northern and far eastern seas of the USSR from Naumov (1960), and those of Alaska from Fraser (1937).

SYSTEMATIC ACCOUNT

Order THECATA (CALYPTOBLASTEA)

Family HALECIIDAE

Halecium curvicaule Lorenz, 1886

Plate I, Fig. 1, 2

Collection records – Stations 13, 226, 333, 61-32, 65-1506.

FIG. 1. Stations of the *Calanus–Salvelinus* expeditions having thecate hydroids.

TABLE 1. *Calanus* and *Salvelinus* stations at which thecate hydroids were collected.

Station	Location	N	W	Date	Depth (*m*)	Bottom	No. species
11	Ungava Bay	59°11′	68°47′	12 VII 47	46	Rock, sand	2
13	"	59 30	69 00	13 VII 47	46–55	–	2
20,21	"	59 53–54	69 29–31	18 VII 47	64	Rock, mud, sand	1
28	"	58 54.5	68 59	19 VII 47	27	Rock	6
33	"	59 13	65 45	8 VIII 47	18–27	Rock, mud	15
58	"	60 05	65 06	28 VII 48	20	–	20
103	"	60 24	64 58	6 VII 49	145–255	Rock	4
106	"	60 21	64 58	7 VII 49	100–110	–	1
109	"	60 23	66 25	16 VII 49	110	–	1
126	"	60 04	69 26	23 VIII 49	70–91	Rock	6
201C	"	59 29.5	65 20.5	29 VI 50	80–100	Mud	5
203	"	58 50	68 18	8 VII 50	30	–	9
206	"	60 45	69 17	15 VII 50	90	Rock	1
208	Hudson Strait	61 05.3	69 34	20 VII 50	80–90	"	2
210	"	61 04.5	69 39	21 VII 50	90–110	"	5
221	"	62 12	69 38	4 VIII 50	274	–	4
225	"	60 40.5	64 39.7	12 VIII 50	22	Rock	6
226	"	60 38	64 38.9	13 VIII 50	90–100	–	14
229	Ungava Bay	59 29	65 17.5	26 VIII 50	0–13	–	3
231	"	59 28.5	65 15	27 VIII 50	63–90	Rock, mud	2
317	Frobisher Bay	62 44	65 29	9 VIII 51	27–36	Rock	3
318	"	62 43	65 38	9 VIII 51	55	–	2
319	"	62 21	65 16	11 VIII 51	113–118	Rock	2
321	"	62 23	64 58	11 VIII 51	71	–	6
322	"	62 46	65 55	12 VIII 51	119	Sand, gravel	1
331	"	62 42	65 31	22 VIII 51	77	–	3
333	"	62 39	65 47.5	24 VIII 51	104–137	–	3
334	"	62 38	65 52	24 VIII 51	183–192	–	6
406	"	62 74	65 26.5	22 VII 52	46	–	5
512	N Hudson Bay	63 27	84 08	8 IX 53	18–21	Rock	1
520	NE Hudson Bay	62 27	79 51	4 VIII 53	73–91	"	4
547	Foxe Channel	63 41	80 12	3 IX 53	73	"	14
608	N Hudson Bay	62 44.5	83 39	9 VIII 54	32	"	2
609	"	62 57.5	82 43	10 VIII 54	18	"	3
611	"	62 59.7	82 39	10 VIII 54	49	Rock, clay	1
616	"	63 15.5	83 41	16 VIII 54	85–90	Rock	21
722	N Foxe Basin	69 20	82 00	29 IX 55	36–45	Mud	1
723	"	69 22.7	81 44.3	26 X 55	22–24	"	1
811	"	69 37.2	81 06.3	17 VIII 56	106	Rock	11
815	"	69 43.2	82 07	18 VIII 56	55	–	1
820	"	69 55.7	80 19	28 VIII 56	73	Mud	1
821	"	70 00.5	80 12	29 VIII 56	0–30	"	2
837	"	69 16	77 48	9 IX 56	45–55	Sand, rock	3
901	"	69 06	79 11	29 VIII 57	0–34	Mud, sand, rock	5

(*Continued*

TABLE 1. *Calanus* and *Salvelinus* stations at which thecate hydroids were collected. (*Concluded*)

Station	Location	N	W	Date	Depth (*m*)	Bottom	No. species
58–33	SE Hudson Bay	57°23′	79°39′	16 IX 58	90–95	Mud, rock	1
59–21	"	56 28	78 04	21 VII 59	36	–	9
59–29	"	55 50	80 15	3 VIII 59	37–40	Rock	5
59–50	"	58 00	79 38	17 VIII 59	50–60	"	9
59–64	"	55 13	82 08	31 VIII 59	23–26	Gravel	8
59–66	"	55 45	82 24	31 VIII 59	70	Mud	4
59–68	"	56 19	82 16	31 VIII 59	100–107	"	2
59–69	"	56 16′	82 42′	31 VIII 59	126	Mud, sand, gravel	1
60–1001	MacKenzie Bay	69 32.5	138 55	26 VII 60	12–37	Mud	2
60–1025	"	69 33	138 55	14 VIII 60	3.5	Mud, debris	1
61–1	W Hudson Bay	60 24	93 55	21 VII 61	60	Rock, mud	8
61–5	NW Hudson Bay	62 30	91 05	25 VII 61	63–71	Rock	3
61–8	"	63 04	90 24	27 VII 61	48–58	"	2
61–10	Chesterfield Inlet	63 43	91 43	30 VII 61	75–90	"	3
61–11	"	63 39	91 27	30 VII 61	20–70	"	5
61–15	Roes Welcome Sound	63 52	88 37	6 VIII 61	45	"	1
61–18	"	63 34	87 20	6 VIII 61	5	–	2
61–24	"	65 07	86 45	9 VIII 61	44–46	Rock	5
61–26	"	65 24	86 44	10 VIII 61	40–75	"	9
61–32	Foxe Basin	66 11	84 12	14 VIII 61	145–160	Mud, rock	1
61–35	Frozen Strait	65 36	83 46	24 VIII 61	155	–	1
61–43	"	66 13	85 09	28 VIII 61	85–92	–	9
61–1015	Liverpool Bay	70 11	128 35	10 VII 61	12	Mud, sand	1
62–019	"	69 49.3	130 19	3 VIII 62	2–10	"	1
62–032	"	69 48	130 19	12 VIII 62	10	"	3
62–2012	Creswell Bay	72 44.6	94 05	25 VII 62	36	Mud, clay	1
62–2014	"	72 44.1	94 06	25 VII 62	35	Clay	2
62–4001	Barrow Strait	74 36	94 13	23 VI 62	35–62	–	4
62–4003	"	74 37.5	94 12	28 VI 62	10–30	Sand, rock	3
62–4011	"	74 37.8	94 18.3	25 VII 62	18	–	1
62–4305	Queens Channel	76 37	96 27.4	16 VII 62	40	–	1
63–004	Franklin Bay	70 06	125 01	4 VIII 63	27–32	Mud	2
63–020	Darnley Bay	69 39.3	123 33.3	12 VIII 63	48–52	Mud, rock	1
63–025	Franklin Bay	69 52	125 54	20 VIII 63	110–130	Mud	1
64–004	Cambridge Bay	69 02.8	105 16.6	6 IX 64	38	–	1
65–1044	Coronation Gulf	68 18.2	109 15	23 VIII 65	120	–	4
65–1502	Strait of Belle Isle	51 26	56 50	30 VII 65	31	–	5
65–1505	"	51 20	56 46	31 VII 65	46–64	–	12
65–1506	"	51 49	56 06	31 VII 65	70–99	–	17
65–1510	"	52 08	55 38	3 VIII 65	55–64	–	1

Description — Colonies creeping or upright, reaching 7 mm high, hydrocaulus monosiphonic. Internodes arising singly or in pairs, curving upward abruptly from their origin just below the hydrothecae and usually terminating distally in a hydrotheca, perisarc thin, smooth, with a bulge above a well-marked node near the proximal end. Internodes typically long and slender in upright colonies, shorter in creeping colonies. Hydrophores occasionally renovated, hydrothecae shallow, 32–53 μ (diaphragm to margin), margin scarcely flaring in some colonies, strongly flaring and often folded over in others, diameter 169–238 μ, diameter at diaphragm 116–163 μ. Punctae large, distinct, forming a ring just distal to the diaphragm.

Female gonophores present only, gonothecae borne on the hydrocaulus, laterally compressed, pear-shaped in broad view, a pair of hydranths issuing from an asymmetrically placed orifice at the apical end, perisarc smooth or slightly wrinkled in some colonies, distinctly corrugated in others.

Remarks — *Halecium curvicaule* has not been recorded from the North American Arctic before, but it has been found at several locations in the Canadian Atlantic (Fraser, 1944). In a detailed study by Dons (1911), *H. curvicaule* was shown to be a variable species, encompassing in its morphology two other described species, *H. mirabile* and *H. repens*. Broch (1918) and most subsequent authors have followed Dons in regarding the latter two species as synonyms of *H. curvicaule*.

Distribution — Northern Canada (new record), west, east Greenland, Iceland, Jan Mayen Island, Spitzbergen, Barents Sea, White Sea, Kara Sea, Laptev Sea.

Halecium groenlandicum Kramp, 1911
Plate I, Fig. 3, 4, 5

Collection records — Station 59-21, 61-1, 61-5, 61-18, 65-1510.

Description — Colonies reaching 3.5 cm high, hydrocaulus and main branches polysiphonic, branching irregular, branches straight or slightly curved but not geniculate, nodes nearly transverse, internodes straight, long, each with several hydrophore-bearing apophyses, apophysis arising from any point on the internode from proximal to distal end, and from any side. Perisarc thick, bulging slightly near the nodes, smooth or slightly wrinkled elsewhere. Hydrophores with a deep basal chamber, often renovated, up to about 7 times, basal chamber of secondary hydrophores of variable length. Hydrothecae shallow, margin flaring very slightly but not everted, diaphragm distinct, punctae small, numerous.

Gonophores and an occasional hydrophore borne on a dense tangle of ramified stolons arising at or near the base of the colony, gonothecae kidney-shaped with a very small aperture on a small process near the distal end, perisarc thin, spines and annulations absent.

Remarks — Collection records suggest that this distinctive species, originally described by Kramp (1911) from northeast Greenland, is fairly common in Hudson Bay. It is previously unreported in North American waters, but its known distribution indicates that it may be circumpolar.

Distribution — Northern Canada (new record), west, east Greenland, Barents Sea, White Sea, Kara Sea, Laptev Sea, Sea of Okhotsk, Bering Sea.

Halecium labrosum Alder, 1859
Plate I, Fig. 6, 7, 8

Halecium labrosum Broch, 1907, p. 5; Fraser, 1922, p. 5; 1931, p. 481; Kramp, 1932b, p. 55; Fraser, 1944, p. 195, fig. 173.

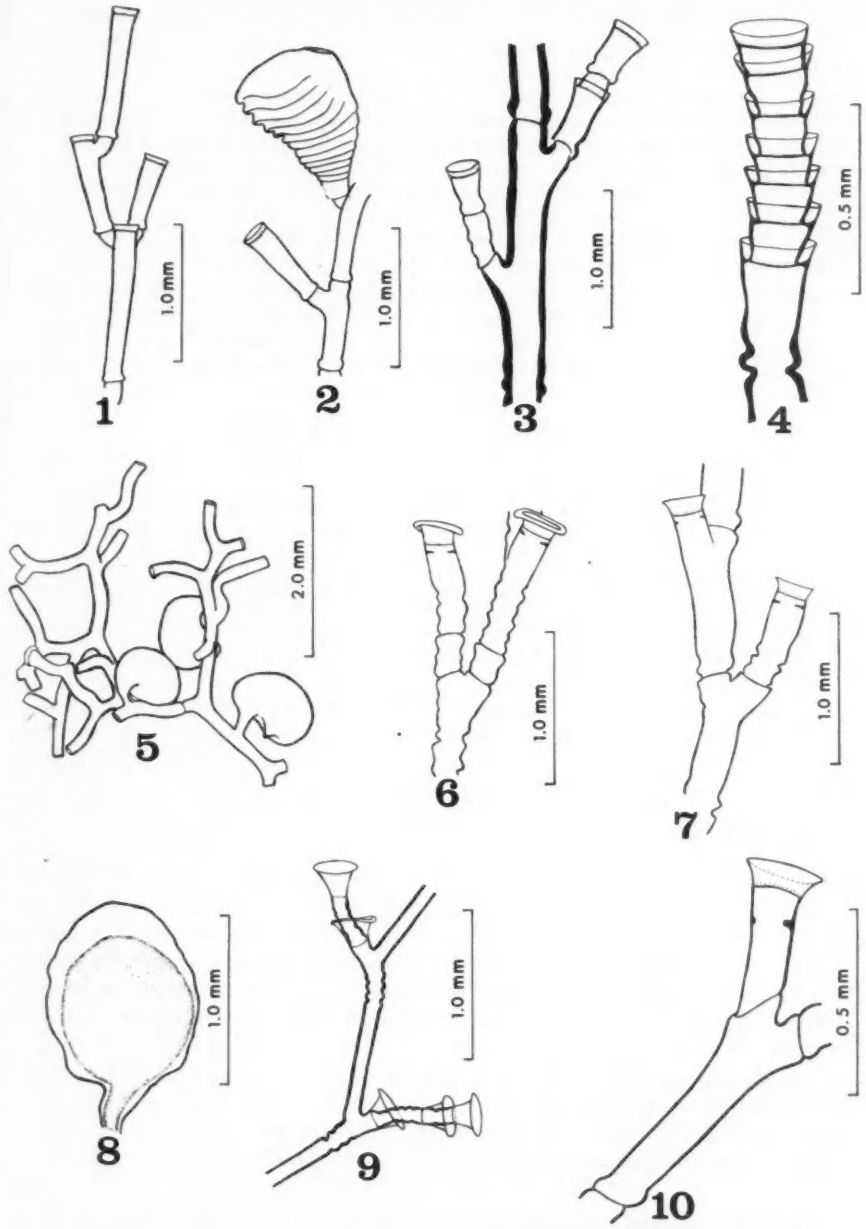

PLATE I. Fig. 1. *Halecium curvicaule*, station (sta.) 33; Fig. 2. *H. curvicaule*, female gonotheca, sta. 333; Fig. 3. *H. groenlandicum*, sta. 61-1; Fig. 4. *H. groenlandicum*, sta. 59-21; Fig. 5. *H. groenlandicum*, gonothecae, sta. 61-1; Fig. 6. *H. labrosum*, sta. 58; Fig. 7. *H. labrosum*, sta. 61-26; Fig. 8. *H. labrosum*, gonotheca, sta. 61-26; Fig. 9. *H. minutum*, sta. 61-43; Fig. 10. *H. muricatum*, sta. 901.

Collection records — Stations 33, 58, 103, 322, 61–26.

Description — Colonies reaching 5.0 cm high, hydrocaulus and main branches polysiphonic, branching irregular. Hydroids varying considerably in morphology from colony to colony, internodes short to fairly long, perisarc thin to fairly thick, annulations typical or hourglass-shaped, degree of internode annulation varying from slightly wrinkled at the proximal end to annulated throughout, branches occasionally geniculate. Apophyses distal on the internode, giving rise to hydrothecae or branches. Hydrophores with deep basal chambers, margin strongly flaring, often distinctly everted. Punctae forming a distinct ring distal to the diaphragm, pseudodiaphragm usually evident.

Gonothecae large, oval, flattened, arising from the branches on short pedicels, perisarc of moderate thickness.

Distribution — Northern Canada, west, east Greenland, Iceland, Faeroes, Spitzbergen, Barents Sea, White Sea, Kara Sea, Chukchi Sea, Sea of Okhotsk, Bering Sea, Alaska.

Halecium minutum Broch, 1903
Plate I, Fig. 9

Collection records — Stations 226, 837, 59-29, 61-11, 61-43, 65-1506.

Description — Colonies reaching 1.3 cm high, hydrocaulus monosiphonic, 95–116 μ in diameter, often geniculate, branching irregular, branches arising just below the hydrothecae, occasionally 2 branches arising at the same height on, but from opposite sides of the hydrocaulus, giving the colonies a dichotomous appearance. Hydrophores often renovated, up to 5 times, hydrothecae deep, cup-shaped, 121–211 μ long (diaphragm to margin), margin flaring, diameter at diaphragm 105–132 μ, diameter at margin 253–316 μ. Punctae present just distal to the diaphragm, some colonies with a pseudodiaphragm immediately below the diaphragm. Hydrocaulus with one or a few hourglass-shaped annulations at the distal end of each internode, smooth elsewhere, secondary hydrophores, if present, annulated basally.

Gonophores not seen.

Remarks — I have not followed Naumov (1960) in regarding this species as a synonym of *H. corrugatum*. In his original description of *H. corrugatum* Nutting (1899) stressed that the pedicels were completely annulated, whereas in Broch's (1903) description of *H. minutum* annulations were few and usually restricted to the distal end of the internodes. Apparent differences also exist in the type of colony formed, *H. minutum* being alternately branched, *H. corrugatum* rarely branched, and in hydrotheca size, those of *H. corrugatum* being small, *H. minutum* relatively large. Further, the gonothecae of *H. corrugatum* as described by Fraser (1937) are distinctly different from those of *H. minutum* described by Broch (1903). *Halecium undulatum* appears to be much more like *H. corrugatum* than does *H. minutum*. *Halecium minutum* has been reported from eastern Canada (Fraser, 1944) but not from the study area before.

Distribution — Northern Canada (new record), west, east Greenland, Iceland, Spitzbergen, Barents Sea, White Sea, Kara Sea, Bering Sea.

Halecium muricatum (Ellis and Solander, 1786)
Plate I, Fig. 10; Plate II, Fig. 1

Halecium muricatum Packard, 1863, p. 403; 1867, p. 264; 1891, p. 369; Whiteaves, 1901, p. 24; Broch, 1907, p. 5; Fraser, 1922, p. 5; 1931, p. 481; Kramp, 1932b, p. 55; Fraser, 1944, p. 197, fig. 176.

Collection records — Stations 33, 58, 226, 547, 616, 901, 61-5, 61-8, 61-43, 65-1502. Also National Museums of Canada (N.M.C.) collection, south of Bartlett Island, Hudson Bay, July 14, 1963, collector A. H. Clarke.

Description — Colonies reaching 5.5 cm high, hydrocaulus and main branches polysiphonic. Branching irregular, branches pinnately branched. Internodes slender, about 4 times longer than wide, perisarc of moderate thickness, bulging slightly near the nodes, smooth elsewhere, nodes oblique, sloping alternately left and right. Hydrophores often renovated, arising from distinct apophyses near the distal end of the internodes, hydrothecae 42–79 μ deep (diaphragm to margin), margin flaring, typically curved to a greater degree on the adcauline side, diameter at margin 179–238 μ, diameter at diaphragm 121–143 μ. Punctae large, distinct, forming a ring just distal to the diaphragm. Pseudodiaphragm conspicuous, usually better developed on the adcauline side.

Gonothecae borne on short pedicels arising from the polysiphonic hydrocaulus and branches, oval in broad outline, spindle-shaped in side view, surface covered with spines.

Distribution — Northern Canada, west, east Greenland, Iceland, Faeroes, Spitzbergen, Barents Sea, White Sea, Kara Sea, Laptev Sea, East Siberian Sea, Chukchi Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

Halecium scutum Clark, 1876
Plate II, Fig. 2, 3

?*Halecium beani* Fraser, 1922, p. 5; 1931, p. 481; 1944, p. 186, fig. 160 (in part).

Collection records — Stations 406, 65-1505.

Description — Colonies reaching 5.5 cm high, hydrocaulus and main branches polysiphonic, branching irregular. Internodes stout, width across distal end, including apophysis, about half the length, perisarc thick, smooth, nodes oblique, sloping alternately left and right. Primary hydrothecae sessile on the apophyses, secondary hydrothecae with fairly large basal chambers. Hydrothecae shallow, margin flaring very slightly but not everted, diaphragm distinct, punctae small, numerous.

Female gonothecae borne on short pedicels arising from the hydrocaulus, oval, surface smooth, perisarc fairly thick, aperture position somewhat variable but always lateral and oblique to the main axis of the gonotheca, margin of the aperture with an adthecal process.

Remarks — This is a robust species similar to but distinguishable from *H. halecinum* and *H. beani* mainly in the shape of the female gonothecae. *Halecium beani* and *H. scutum* differ from *H. halecinum* in having a lateral rather than a distal gonothecal aperture. *Halecium scutum* differs from *H. beani* in having the aperture oblique to, rather than approximately parallel with the main axis of the gonotheca, and in having an adthecal lingueform protuberance on the aperture margin (Broch, 1918). Broch, who reidentified as *H. scutum* several specimens that had previously been identified as *H. beani*, believed that the relatively warmwater *H. beani* is absent in the Arctic. Fraser's (1922, 1931, 1944) reports of *H. beani* from King George's Sound in Hudson Strait were probably based on *H. scutum*. The brief description of the species in his 1944 monograph could apply to *H. scutum* as well as to *H. beani*.

While recognizing *H. beani* and *H. scutum* as being very similar, there seems to be insufficient basis to regard them as conspecific, as Naumov (1960) does.

Distribution — Northern Canada (new record), west Greenland, Iceland, Faeroes, Spitzbergen, Barents Sea, White Sea, Kara Sea, Laptev Sea, Chukchi Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

Halecium speciosum Nutting, 1901
Plate II, Fig. 4

Collection records — Stations 28, 608, 811, 61-1.

Description — Colonies reaching 2.6 cm high, hydrocaulus and main branches polysiphonic, at least in older colonies, branching irregular, branches geniculate. Internodes arising singly or in pairs from the previous internode just below the hydrotheca, internodes straight, typically long and slender, perisarc of moderate thickness, annulated proximally, smooth distally and terminating in a hydrotheca. Hydrophores occasionally renovated 1–2 times, hydrothecae 42–53 μ deep (diaphragm to margin), margin flaring slightly but not everted, diameter at margin 195–232 μ, at diaphragm 169–190 μ. Punctae large, distinct, forming a ring just distal to the diaphragm.

Male gonophores only present, gonothecae elongate, somewhat asymmetrical, arising just below the hydrothecae from short pedicels, perisarc fairly thin, surface varying from practically smooth or slightly wavy to distinctly furrowed transversely, being more deeply furrowed on one side.

Remarks — These specimens very closely resembled hydroids from Spitzbergen identified as *H. ornatum* by Broch (1910), but Broch's description and figures indicate that his specimens were probably *H. speciosum*. The original description of *H. ornatum* by Nutting (1901) was rather meagre, but the species evidently differs from *H. speciosum* in: (1) being much less annulated; (2) having a more everted hydrothecal margin; (3) having less distinct internodes that do not necessarily arise below the hydrotheca of the preceding internode. In these characters Broch's hydroids and those of the *Calanus* expedition resembled *H. speciosum*. Apparent differences in the shape of the gonothecae appear inconsequential; Nutting (1901) believed mature gonothecae of *H. ornatum* would resemble *H. speciosum*.

Distribution — Northern Canada (new record), Spitzbergen, Sea of Okhotsk, Bering Sea, Alaska.

Halecium undulatum Billard, 1921
Plate II, Fig. 5–9

Halecium tenellum Fraser, 1931, p. 481.

?*Halecium tenellum* Verrill, 1879, p. 152; Fraser, 1922, p. 5; 1944, p. 201, fig. 179 (in part).

Collection records — Stations 331, 333, 520, 616, 59-21, 59-64, 59-66, 65-1506. Also N.M.C. collection, Wakeham Bay, washed ashore on *Laminaria*, October 1927, collector F. Johansen.

Description — Colonies reaching 1.5 cm high, hydrocaulus monosiphonic, often geniculate, 84–143 μ wide, branching irregular, branches arising just below the hydrothecae, occasionally two branches arising at the same height on, but from opposite sides of the hydrocaulus, giving some colonies a dichotomous appearance. Colonies varying considerably in degree of annulation, in some colonies perisarc smooth except for one or a few annulations at the distal end of each internode, in others perisarc annulated throughout, annulations typically hourglass-shaped, perisarc varying from thin to thick. Hydrophores often renovated, up to about 5 times, hydrothecae 42–105 μ deep (diaphragm to margin), saucer-shaped, margin strongly flaring, often folded over, diameter at margin 185–240 μ, at diaphragm 95–116 μ. Punctae large, distinct, forming a ring just distal to the diaphragm, pseudodiaphragm occasionally present.

Gonothecae large, oval, flattened, 1.6–2.5 mm high, 1.3–1.6 mm wide in broad view, arising by a short pedicel from the stolon network. Surface smooth except for two rounded spines at the apex.

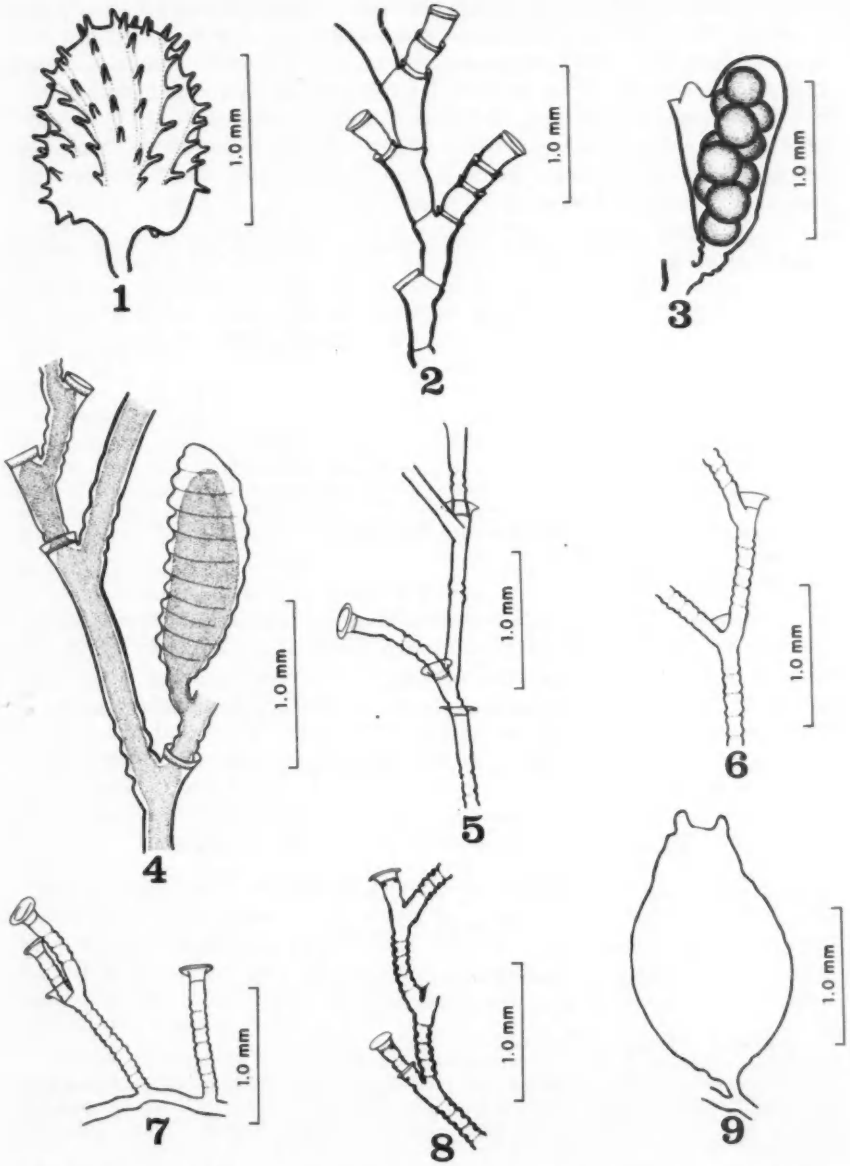

PLATE II. Fig. 1. *Halecium muricatum*, gonotheca, sta. 901; Fig. 2. *H. scutum*, sta. 65-1505; Fig. 3. *H. scutum*, gonotheca, sta. 65-1505; Fig. 4. *H. speciosum*, sta. 811; Fig. 5. *H. undulatum*, sta. 333; Fig. 6. *H. undulatum*, Wakeham Bay; Fig. 7. *H. undulatum*, sta. 59-64; Fig. 8. *H. undulatum*, Wakeham Bay; Fig. 9. *H. undulatum*, gonotheca, Wakeham Bay.

Remarks — This species is probably more widely distributed than present records indicate. Hamond (1957) showed that numerous arctic and subarctic records of *H. tenellum* were actually based on specimens of *H. undulatum*, and he believed the former to be a more southern species than the latter. None of the *Halecium* hydroids from northern Canada approached the small size of *H. tenellum* as described by Millard (1957) and Vervoort (1959, 1966). Specimens from Wakeham Bay identified by Fraser (1931) as *H. tenellum* were examined and reidentified as *H. undulatum*.

Distribution — Northern Canada (new record), west Greenland, Iceland, Faeroes, northern seas of the USSR (?).

Family LAODICEIDAE

Ptychogena lactea A. Agassiz, 1865

Plate III, Fig. 1

Collection records — Station 65-1044.

Description — Colony consisting of a creeping hydrorhiza with upright, spirally coiled, unbranched pedicels of variable length, pedicels merging imperceptably with the base of the hydrotheca, diaphragm absent. Hydrothecae smooth, long and tubular, expanding gradually toward the margin, occasionally somewhat curved, margin flaring slightly, often renovated. Operculum cone-shaped, occasionally inverted, consisting of a folded membrane, distinctly marked off from the hydrotheca.

Gonothecae not seen.

Remarks — The hydroids described here are inseparable from Naumov's (1960) account of the polyp *Ptychogena lactea* from the northern USSR. The identity of the hydroid is unsubstantiated by life-history studies, but Naumov expressed little doubt that it was the polyp of the circumpolar medusa *P. lactea*. Naumov's hydroids were collected at depths of 255 and 520 m in the Barents and Kara seas, where the only metagenetic medusa at such depths was *P. lactea*. The medusa of this species was also reported by Grainger (1965) from the south Beaufort Sea, not far from the hydroid locality reported here. This is the first report of the hydroid outside Soviet waters.

Distribution — Northern Canada (new record), Barents Sea, Kara Sea.

'INCERTAE SEDIS

Cuspidella humilis Hincks, 1866

Plate III, Fig. 2

Collection records — Stations 608, 611, 616, 811, 58–33.

Description — Colony consisting of a creeping stolon with sessile, cylindrical hydrothecae, hydrothecae reaching 530 μ long, 115 μ wide, given off at right angles to the stolon, constricted slightly at the base, diaphragm indiscernible, operculum formed by a folded continuation of the hydrothecal wall.

Gonothecae not seen.

Remarks — *Cuspidella humilis* and *C. grandis* were distinguished by Hincks (1868) on the basis of size, but no measurements were given and de-

termining which species a given hydroid belongs to is left to a subjective judgment by the identifier. Measurements subsequently published for the two species have not always been in accord. Fraser's (1944) *C. grandis*, for example, is little larger than Naumov's (1960) *C. humilis* (the polyp of *Staurophora mertensi*). The present specimens, although larger than Vervoort's (1968) material of the same species, are regarded as *C. humilis*, agreeing closely with the descriptions of Broch (1918) and Naumov (1960). However, whereas the hydroid from Soviet waters was linked by Naumov (1951) to the medusa *S. mertensi*, I hesitate to identify the hydroid from *Calanus* collections as *S. mertensi* because Rees and Russell (1937) found the polyp of *Mitrocomella brownei*, a *Cuspidella*, to resemble *C. humilis* also. Life-history studies have demonstrated that a number of medusoid genera in several families possess *Cuspidella* hydroids (Table 2). Thus it appears impossible to ascertain from

TABLE 2. List of medusae known or suspected of having *Cuspidella* hydroids.

Medusa	Hydroid	References
Dipleurosoma typicum	*?Cuspidella* sp. [a]	Browne (1900), Russell (1953)
Laodicea undulata	*Cuspidella* sp.	Russell (1936, 1953)
Staurophora mertensi	*Cuspidella humilis*	Naumov (1951, 1960)
Cosmetira pilosella	*Cuspidella* sp.	Rees (1941)
Mitrocoma annae	*Cuspidella*-like	Metschnikoff (1886), Russell (1936)
Mitrocomella brownei	*Cuspidella* sp.	Rees and Russell (1937)
Tiaropsis multicirrata	*Cuspidella mollis*	Korsakova (1949), Naumov (1951, 1960)
Cirrholovenia tetranema	*Cuspidella* sp.	Brinckmann (1965)

[a]Confirmation needed.

present knowledge of the polyp to which medusoid genus or species a given *Cuspidella* hydroid belongs. Moreover, the polyp of a given medusoid species may vary considerably in morphology. Russell (1936) found that the hydroid of *Laodicea undulata* varied from small forms like *C. humilis* and *C. costata* to an elongate form like *C. grandis*, and even a partially recumbent form resembling *C. procumbens*.

While recognizing *Cuspidella* as a junior synonym of several recognized medusoid genera, the name must be retained at least temporarily for classifying *Cuspidella* hydroids of uncertain affinity. As life histories become better known, hydroids of the genus may then be placed in the genus of the corresponding medusa.

Distribution — Northern Canada (new record), west, east Greenland, Iceland, Faeroes, Spitzbergen, northern USSR.

Cuspidella procumbens Kramp, 1911
Plate III, Fig. 3

Collection records — Stations 811, 59-68.

Description — Hydrothecae sessile, smooth, cylindrical, adnate to the stolon for a varying distance, free portion curving upward, distal portion nearly perpendicular to the substrate, 463–662 μ long, 99–146 μ wide, diaphragm indiscernible. Operculum a continuation of the hydrothecal wall, with about 8 facets when folded.

Gonothecae not seen.

Distribution — Northern Canada (new record), west, east Greenland.

Family PHIALELLIDAE
Opercularella lacerata (Johnston, 1847)
Plate III, Fig. 4

Opercularella lacerata Fraser, 1922, p. 5; 1931, p. 481; 1944, p. 176, fig. 150.

Collection records — Stations 723, 901, 65-1044. Also N.M.C. collection, south of Herschel Island, 4–6 m, August 11, 1960, collector D. E. McAllister.

Description — Colony reaching about 8 mm high, hydrocaulus monosiphonic, annulated or wrinkled throughout, branching irregular. Hydrothecae turbinate, not distinctly demarcated from the pedicel, operculum conical, consisting of a folded continuation of the hydrothecal wall, with about 9–10 facets when folded, pedicels annulated throughout in a close spiral.

Gonothecae not seen.

Distribution — Northern Canada, west Greenland, Iceland, Faeroes, Barents Sea, White Sea.

Family CALYCELLIDAE
Calycella gracilis Hartlaub, 1897
Plate III, Fig. 5, 6

Collection records — Station 229.

Description — Colony with a creeping stolon having upright, unbranched pedicels of variable length, reaching 1.7 mm long, annulated throughout in a close spiral. Hydrothecae cylindrical, 475–844 μ long, 111–147 μ wide at the margin, diaphragm absent. Operculum with about 10 facets having delicate folds between each adjacent pair, separated from the hydrothecal wall by a distinct scalloped edge, margin not flaring, often renovated.

Male gonophores present only, gonophores fixed, sperm developing around a distinct spadix. Gonothecae arising from the stolon by a short annulated pedicel, smooth, fusiform, 433–675 μ long, 159–206 μ wide in the midregion.

Remarks — This is the first record of this species since its original description from Helgoland by Hartlaub (1897). A similar hydroid was found in Britain by Hamond (1957) but its specific identity was not determined. In the *Calanus–Salvelinus* collections the only record was from eastern Ungava Bay where colonies were present on the bryozoan *Microporina articulata*. The gonophores, previously unknown, are sporosacs, confirming Hartlaub's opinion that the hydroid belongs to the genus *Calycella*.

Hydrothecae of *C. gracilis* are similar to those of the more common *C. syringa* but are longer, relatively more slender, and do not flare at the margin.

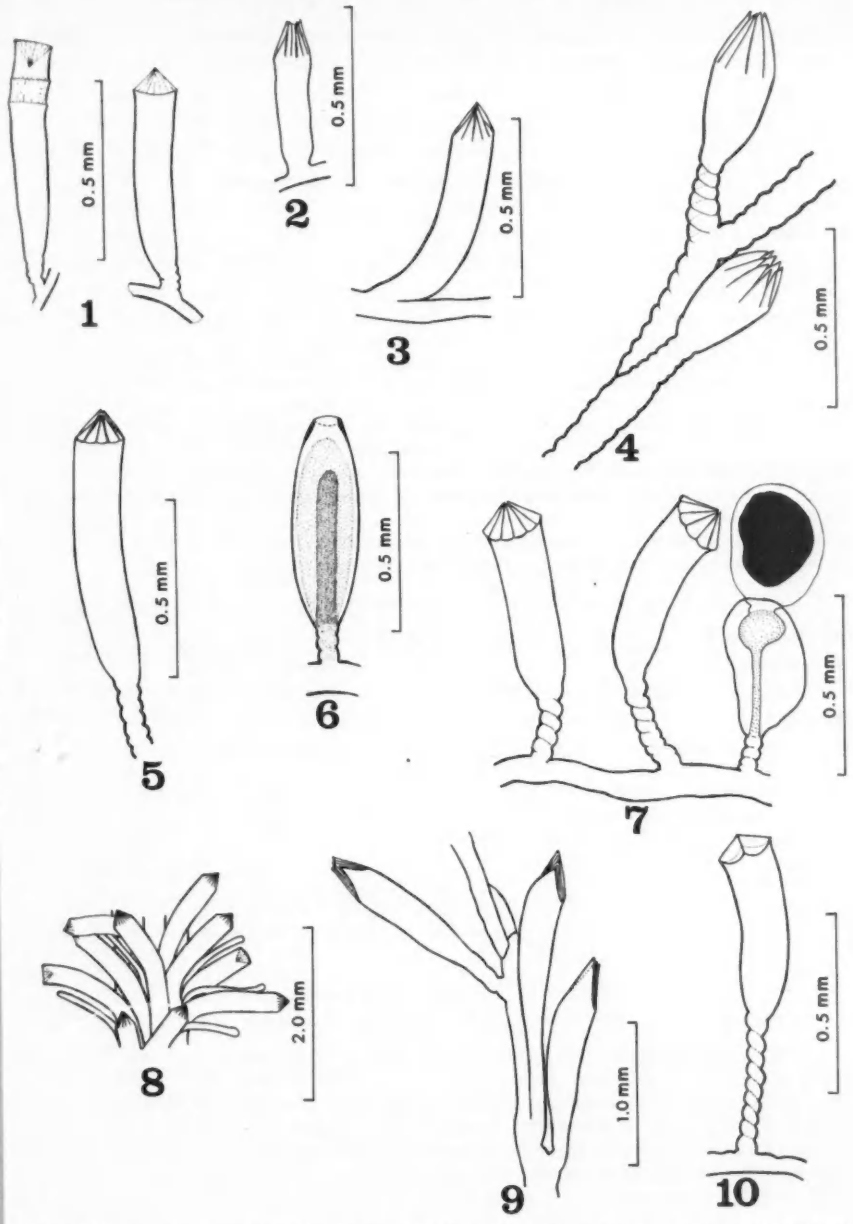

PLATE III. Fig. 1. *Ptychogena lactea*, sta. 65-1044; Fig. 2. *Cuspidella humilis*, sta. 611; Fig. 3. *C. procumbens*, sta. 811; Fig. 4. *Opercularella lacerata*, S of Herschel Is., N.W.T.; Fig. 5. *Calycella gracilis*, sta. 229; Fig. 6. *C. gracilis*, gonotheca, sta. 229; Fig. 7. *C. syringa*, sta. 65-1505; Fig. 8. *Lafoeina maxima*, sta. 62-4001; Fig. 9. *Stegopoma plicatile*, S of Bartlett Is., Hudson Bay; Fig. 10. *Tetrapoma quadridentatum*, sta. 65-1506.

The gonothecae of the two species are distinctly different in morphology, those of *C. syringa* being short and oval or club-shaped, those of *C. gracilis* being relatively long and fusiform.

Distribution — Northern Canada (new record).

Calycella syringa (Linnaeus, 1767)
Plate III, Fig. 7

Calycella syringa Hincks, 1874, p. 148; Broch, 1907, p. 7; Fraser, 1922, p. 4; 1931, p. 480; 1939, p. 59; 1944, p. 166, fig. 138.

Collection records — Stations 11, 20, 21, 28, 33, 58, 126, 201C, 203, 221, 225, 226, 317, 318, 321, 406, 512, 547, 616, 811, 901, 59-21, 59-29, 59-50, 59-64, 61-1, 61-11, 61-24, 61-43, 62-2014, 62-4001, 65-1505, 65-1506. Also N.M.C. collections, mouth of Lake Harbour, Baffin Island, 55 m, August 4, 1939; south of Bartlett Island, Hudson Bay, July 14, 1963, collector A. H. Clarke.

Description — Colony with a creeping stolon and upright, unbranched pedicels of varying length, annulated throughout in a close spiral. Hydrothecae cylindrical, often somewhat curved, 278–563 μ long (base to margin), 119–212 μ wide at the margin, diaphragm absent. Operculum with about 9–13 facets when folded, separated from the hydrothecal wall by a scalloped edge, margin flaring slightly, often renovated.

Gonophores fixed, mature gonangia with acrocysts. Gonothecae oval or club-shaped, smooth, borne on short annulated pedicels arising from the stolon.

Remarks — This hydroid occurred at more stations (35) than any other species. As Broch (1918) noted, the operculum is easily lost in this species, occasionally complicating its identification. Determination of such specimens is usually facilitated by the presence of entire hydrothecae on the same stolon system, but even in the absence of an operculum a careful examination of the hydrotheca shape will normally establish its identity.

Distribution — Northern Canada, west, east Greenland, Iceland, Faeroes, Spitzbergen, Barents Sea, White Sea, Kara Sea, Laptev Sea, East Siberian Sea, Chukchi Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

Lafoeina maxima Levinsen, 1893
Plate III, Fig. 8

Lafoeina maxima Broch, 1907, p. 3.

Meganema claviformis Fraser, 1939, p. 60, fig. 2.

Collection records — Stations 616, 59-50, 61-26, 62-032, 62-4001, 62-4003, 62-4011.

Description — Colonies large, clavate, consisting of an upright polysiphonic rhizocaulus nearly uniform in diameter throughout, reaching 5.5 cm high, 4 mm wide, unbranched. Hydrothecae sessile, cylindrical, closely set, curving outward from the rhizocaulus. Operculum a cone-shaped lid, occasionally inverted, formed by a folded continuation of the hydrothecal wall. Nematothecae numerous, often reaching outward the same distance as the hydrothecae but much more slender, with a battery of nematocysts near the tip.

Gonothecae not seen.

Remarks — Fraser (1939) described a new hydroid genus and species, *Meganema claviformis*, from Dolphin and Union Strait, N.W.T. The genus *Meganema* was placed in synonymy with *Lafoeina* by Fraser (1946) but he

maintained that *L. claviformis*, though similar to *L. maxima*, was a valid species. The characters used by Fraser to distinguish the two vary considerably within a single colony, and *L. claviformis* is regarded as a synonym of *L. maxima*.

A great diversity of operculum construction exists in campanulinid hydroids, as Kramp (1911) showed. Despite Kramp's detailed descriptions showing that the operculum in *Lafoeina*, *Cuspidella*, "*Campanulina*," and *Opercularella* consists of a folded continuation of the hydrothecal wall, in some subsequent papers the operculum is described as being composed of separate plates or segments. The operculum of *Calycella* is also a folded cylindrical membrane but differs in being separated from the hydrotheca by a scalloped edge, and is formed from the periphery of the hydrothecal roof rather than the hydrothecal wall. In *Tetrapoma* the operculum is formed from the original hydrothecal roof as well, but the inner folds become split off so that the covering actually does consist of separate plates. Fully developed hydrothecae of *Lovenella* have an operculum of separate plates also.

Distribution — Northern Canada, west, east Greenland, Iceland, Spitzbergen, Barents Sea, Kara Sea, Laptev Sea, East Siberian Sea, Chukchi Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

Stegopoma plicatile (M. Sars, 1863)
Plate III, Fig. 9

Stegopoma plicatile Fraser, 1931, p. 481; Kramp, 1932b, p. 27; Fraser, 1944, p. 179, fig. 154.

Collection records — Station 334. Also N.M.C. collection, south of Bartlett Island, Hudson Bay, July 14, 1963, collector A. H. Clarke.

Description — Colonies reaching 3.5 cm high, hydrocaulus and branches polysiphonic except at the extremities. Hydrothecae large, fusiform, usually curved, free from the hydrocaulus and supported by a short pedicel, or with the adaxial wall adnate to the hydrocaulus for a varying distance, diaphragm absent. Margin with two large teeth, operculum in the shape of a rooflike lid.

Gonophores not seen.

Distribution — Northern Canada, west, east Greenland, Iceland, Spitzbergen, Barents Sea, white Sea, Kara Sea, Laptev Sea, Sea of Okhotsk, Sea of Japan, Bering Sea.

Tetrapoma quadridentatum (Hincks, 1874)
Plate III, Fig. 10

Collection records — Stations 58, 103, 226, 406, 547, 616, 61-26, 65-1506.

Description — Colony with a creeping stolon and upright, unbranched pedicels of varying length, spirally annulated throughout. Hydrothecae cylindrical, often somewhat curved, 384–596 μ long (base to margin), 172–192 μ wide at the margin, diaphragm absent. Operculum of four plates, each hinged basally, margin flaring slightly, occasionally renovated, with four teeth.

Gonothecae not seen.

Remarks — Although this hydroid resembles *Calycella syringa* it is distinguishable in having four marginal teeth and four opercular plates. The only other report of this species in North American waters is Stafford's (1912)

record from Gaspé, Que., in the Gulf of St. Lawrence. It was originally described from Frederikshaab, Greenland, by Hincks (1874), who at first believed the material was from Iceland (Kramp, 1938). The species has not been reported from Iceland or the Faeroes but the present records indicate that it penetrates into the subarctic.

Distribution — Northern Canada (new record), west, east Greenland, Spitzbergen, Barents Sea, White Sea, Kara Sea, East Siberian Sea, Sea of Okhotsk.

Family CAMPANULARIIDAE

Campanularia groenlandica Levinsen, 1893

Plate IV, Fig. 1

Collection records — Stations 33, 58, 203, 547, 616, 59-50, 65-1505, 65-1506.

Description — Colony consisting of a creeping stolon and upright, unbranched pedicels reaching 5 mm long, annulated or wrinkled throughout. Hydrothecae campanulate, 935–1019 μ long, 585–668 μ wide, distinct annular thickening basally, basal chamber small, margin with about 14 deeply cut, rounded teeth, with a distinct line originating between each adjacent pair of teeth and running down the hydrothecal wall. Hydrotheca somewhat polygonal in transverse section.

Gonothecae 1920–2322 μ long, 566–952 μ wide, arising via short, smooth pedicels from the stolon, flask-shaped, passing gradually into a long tubular neck.

Remarks — Although *C. groenlandica* is presumably a circumpolar species (Broch, 1918), hitherto the most northerly records of the hydroid in Canada were from the vicinity of the Queen Charlotte Islands in the Pacific (Fraser, 1936) and from Cheticamp Island (Fraser, 1927) in the Atlantic. *Campanularia groenlandica* was originally described near Greenland in Davis Strait by Levinsen (1893).

Distribution — Northern Canada (new record), west, east Greenland, Iceland, Faeroes, Spitzbergen, Barents Sea, White Sea, Kara Sea, Laptev Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

Campanularia integra MacGillivray, 1842

Plate IV, Fig. 2

Campanularia integra Hincks, 1868, p. 163; Broch, 1907, p. 6; Fraser, 1931, p. 480; Kramp, 1932b, p. 63; Fraser, 1944, p. 122, fig. 94.

Campanularia caliculata Verrill, 1879, p. 16; Whiteaves, 1901, p. 23.

Eucopella caliculata Fraser, 1944, p. 146, fig. 119.

Collection records — Stations 28, 58, 203, 221, 225, 317, 321, 331, 547, 616, 811, 59-21, 61-11, 61-24, 62-4001, 62-4003, 62-4305. Also N.M.C. collection, Wakeham Bay, washed ashore on *Laminaria*, October 1927, collector F. Johansen; mouth of Lake Harbour, Baffin Island, 55 m, August 4, 1939.

Description — Colony consisting of a creeping stolon and upright, unbranched pedicels reaching about 4 mm long, wrinkled or spirally annulated throughout, or with a few annulations at each end and smooth in the middle, a ball-shaped annulation invariably present at the hydrothecal base. Hydrothecae campanulate, 618–1002 μ long, 434–701 μ wide, margin entire, somewhat flaring, annular thickening basally. Hydrothecal wall varying from thin to very thick.

Gonothecae 835–2255 μ long, 568–752 μ wide, arising via a short pedicel from the stolons, variable in shape, strongly ribbed spirally.

Remarks — *Campanularia integra* and *C. caliculata* are regarded as synonyms here because of convincing studies by Broch (1918), who found transitional forms between the two on the same stolons. Otherwise the two appear quite distinct, the typical *C. integra* having larger hydrothecae and larger, long, and relatively slender gonothecae containing sporosacs, the form *C. caliculata* having smaller hydrothecae, smaller, short, and stout gonothecae containing eumedusoids. Hydroids of the form *C. caliculata* were found at stations 28, 203, 61-11, and 62-4305; the remaining records were based on specimens of the typical form. Naumov (1960) reported that *C. integra* forma *caliculata* is typical of areas subjected to heavy surf or strong currents.

Distribution — Northern Canada, west, east Greenland, Iceland, Faeroes, Jan Mayen Island, Spitzbergen, Barents Sea, White Sea, Kara Sea, Laptev Sea, Chukchi Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

Campanularia speciosa Clark, 1876
Plate IV, Fig. 3

Campanularia speciosa Fraser, 1931, p. 480; 1944, p. 128, fig. 101.

Collection records — Stations 58, 210, 225, 226, 547, 616, 59-50, 62-2014, 65-1505, 65-1506.

Description — Colony with a creeping stolon and upright, unbranched pedicels usually less than 4 mm long, annulated or wrinkled throughout. Hydrothecae large, elongate, vase-shaped, narrow near the middle, expanded basally and at the margin, 1.65–2.05 mm long, 580–650 μ wide at the margin, margin with 8–9 rounded, shallow teeth, each with a distinct line running from its apex a short distance down the hydrotheca. Hydrotheca with an annular thickening basally, basal chamber small.

Gonothecae large, 2.25–2.67 mm long, 0.84–0.90 mm wide, borne on the stolon, oval, irregularly and indistinctly rugose with a tubular neck of varying length.

Remarks — This large, distinctive campanularian was first described by Clark (1876a) from Alaska. Later, Fraser (1913) described as *C. magnifica* a hydroid from Nova Scotia that differed principally from *C. speciosa* in the morphology of the gonotheca. Broch (1918) showed that the differences used to distinguish the two were based merely on different stages of gonotheca development.

Distribution — Northern Canada, west Greenland, Spitzbergen, Barents Sea, Kara Sea, Laptev Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

Campanularia verticillata (Linnaeus, 1758)
Plate IV, Fig. 4

Campanularia verticillata Packard, 1863, p. 404; 1867, p. 265; 1891, p. 370; Fraser, 1931, p. 480; Kramp, 1932b, p. 64; Fraser, 1944, p. 129, fig. 103.

Collection records — Stations 33, 334, 520, 547, 61-35, 65-1502.

Description — Colony consisting of an upright, irregularly branched, polysiphonic rhizocaulus reaching 6.5 cm in height, nearly the same diameter throughout, terminating in a stump. Pedicels given off in verticils, either annulated throughout or smooth in the middle and annulated at both ends. Hydrothecae variable in size, 835–1453 μ long, 384–668 μ wide, margin with rounded

teeth, varying considerably in shape, from shallow to deeply cut, 12–14 in number. Hydrothecae with a distinct annular thickening basally, basal chamber small.

Gonothecae borne on the rhizocaulus, 1837–2255 μ long, 701–802 μ wide, fusiform with a long tubular neck.

Distribution — Northern Canada, west, east Greenland, Iceland, Faeroes, Spitzbergen, Barents Sea, White Sea, Kara Sea, Laptev Sea, East Siberian Sea, Chukchi Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

Campanularia volubilis (Linnaeus, 1758)
Plate IV, Fig. 5

Clytia volubilis Packard, 1867, p. 265; 1891, p. 369.

Campanularia volubilis Fraser, 1922, p. 4; 1931, p. 480; 1933, p. 566; 1944, p. 131, fig. 104.

Collection records — Stations 28, 33, 58, 103, 126, 201C, 203, 225, 226, 317, 321, 334, 406, 547, 609, 616, 59-29, 59-50, 59-64, 61-1, 61-11, 61-24, 61-26, 61-43, 65-1044, 65-1505, 65-1506.

Description — Colony consisting of a creeping stolon and upright, unbranched pedicels reaching 5 mm in length, spirally annulated throughout except at the hydrothecal base where a ball-shaped annulation occurs. Hydrothecae deeply campanulate to cylindrical, 463–662 μ long, 198–351 μ wide, distinct annular thickening basally, margin often renovated, with 10–15 shallow, rounded teeth.

Gonothecae borne on the stolons, 918–1420 μ long, 351–501 μ wide, oval to elongate-oval with a long tubular neck.

Remarks — I concur with Naumov (1960) that *C. urceolata* Clark, 1876 is a synonym of this species. There is nothing in Clark's (1876a) original description, or in later descriptions of *C. urceolata* (Torrey, 1902; Nutting, 1901, 1915; Fraser, 1937, 1944) to distinguish it from *C. volubilis*. Fraser (1944) separated the two on the basis of teeth number (about 10 in *C. volubilis*, 12–18 in *C. urceolata*) and hydrothecal shape (tubular in *C. volubilis*, variable in *C. urceolata*). However, Broch (1918) found an overlap in teeth number, reporting 9–13 in *C. volubilis*, and Naumov (1960) found an overlap in hydrothecal shape.

Distribution — Northern Canada, west, east Greenland, Iceland, Faeroes, Jan Mayen Island, Spitzbergen, Barents Sea, White Sea, Kara Sea, Laptev Sea, East Siberian Sea, Chukchi Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

Gonothyraea loveni (Allman, 1859)
Plate IV, Fig. 6

Gonothyraea loveni Fraser, 1922, p. 4; 1931, p. 480; 1944, p. 149, fig. 123.

Laomedea hyalina Kramp, 1932b, p. 66.

Collection records — Stations 33, 58, 226, 231, 616, 901, 59-21, 59-50, 62-032, 63-004, 65-1505.

Description — Colonies reaching 5 cm high, hydrocaulus monosiphonic, horn-coloured, rather geniculate, branching regular, arising from all sides of the hydrocaulus in larger colonies. Hydrothecae variable in shape and size, funnel-shaped to nearly cylindrical, 601–1002 μ long, 301–468 μ wide, margin with 14–15 distinct teeth, truncate distally or faintly notched. Diaphragm thin, basal chamber of moderate size.

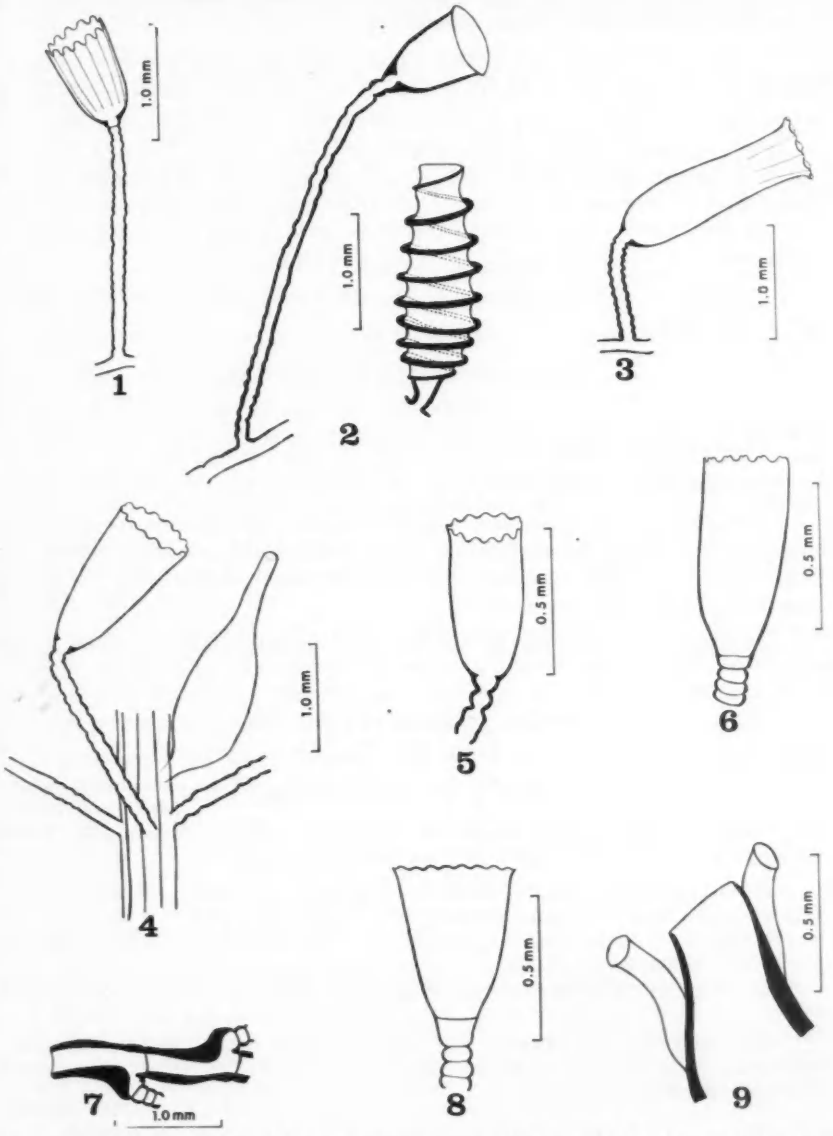

PLATE IV. Fig. 1. *Campanularia groenlandica*, sta. 65-1505; Fig. 2. *C. integra*, hydrotheca, sta. 58; gonotheca, sta. 61-24; Fig. 3. *C. speciosa*, sta. 65-1505; Fig. 4. *C. verticillata*, sta. 547; Fig. 5. *C. volubilis*, sta. 65-1505; Fig. 6. *Gonothyraea loveni*, sta. 65-1505; Fig. 7. *Obelia geniculata*, hydrocaulus, sta. 609; Fig. 8. *O. longissima*, sta. 60-1001; Fig. 9. *Filellum serpens*, on *Abietinaria abietina*, sta. 65-1506.

Gonothecae axillary, cone-shaped, 668–1169 μ long, 434–585 μ wide, terminal collar absent. Ova and sperm developing within meconidia.

Remarks — The genus *Gonothyraea* is recognized here for reasons stated previously (Calder, 1970). Nutting (1901), Fraser (1944), and Naumov (1960) have been followed in regarding *G. hyalina* Hincks, 1866 as a synonym of *G. loveni* although Broch (1918), Kramp (1932b), and Vervoort (1946) have separated them. Broch noted that the two species were very similar but distinguished *G. hyalina* by its teeth, which are occasionally bimucronate and by the longitudinal lines running from the teeth a short distance down the hydrotheca.

Distribution — Northern Canada, west Greenland, Iceland, Faeroes, Spitzbergen, Barents Sea, White Sea.

Obelia geniculata (Linnaeus, 1758)
Plate IV, Fig. 7

Obelia geniculata Verrill, 1879, p. 16; Fraser, 1944, p. 158, fig. 130.

Collection records — Station 609.

Remarks — These specimens were in too poor a condition to adequately describe, the preservative having evaporated. Although the hydrothecae were distorted beyond recognition, the characteristic morphology of the hydrocaulus facilitated identification.

Distribution — Northern Canada, west Greenland, Iceland, Faeroes, Barents Sea, White Sea.

Obelia longissima (Pallas, 1766)
Plate IV, Fig. 8

Obelia longissima Fraser, 1931, p. 480; 1944, p. 162, fig. 133.

Collection records — Stations 203, 60-1001. Also N.M.C. collection, Wakeham Bay, washed ashore on *Laminaria*, October 1927, collector F. Johansen.

Description — Colony reaching 10 cm high, hydrocaulus monosiphonic, dark brown, somewhat geniculate, branching regular, alternate, branches arising from all sides of the hydrocaulus. Hydrothecae funnel-shaped, 585–601 μ long, 301–412 μ wide, margin entire or wavy, diaphragm thin, basal chamber large.

Gonothecae axillary, club-shaped, 1169–1720 μ long, 384–518 μ wide, with a small terminal collar.

Distribution — Northern Canada, west, east Greenland, Iceland, Faeroes, Spitzbergen, Barents Sea, White Sea, Kara Sea, Laptev Sea, East Siberian Sea, Chukchi Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

Family LAFOEIDAE
Filellum serpens (Hassall, 1848)
Plate IV, Fig. 9

Filellum serpens Fraser, 1922, p. 5; 1931, p. 481; 1944, p. 215, fig. 198.

Collection records — Stations 33, 58, 126, 201C, 226, 811, 59-64, 61-10, 65-1506. Also N.M.C. collection, south of Bartlett Island, Hudson Bay, July 14, 1963, collector A. H. Clarke.

Description — Hydrothecae sessile, cylindrical, curving upward from a creeping stolon. Adnate portion of the hydrothecae smooth, margin entire, flaring very slightly, opening diameter 113–146 μ.

Coppinia not seen.

Distribution — Northern Canada, west, east Greenland, Iceland, Faeroes, Spitzbergen, Barents Sea, White Sea, Kara Sea, Laptev Sea, East Siberian Sea, Chukchi Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

Grammaria abietina (M. Sars, 1851)
Plate V, Fig. 1

Grammaria abietina Broch, 1907, p. 7; Kramp, 1932b, p. 34; Fraser, 1944, p. 217, fig. 200.

Collection records — Stations 210, 520, 547, 811, 65-1506.

Description — Colony reaching 10 cm high, consisting of an upright, irregularly branched, polysiphonic rhizocaulus. Branches polysiphonic throughout, terminating abruptly, constricted basally but elsewhere nearly the same diameter as the rhizocaulus. Hydrothecae tubular, arranged in six longitudinal series on both stems and branches, curving outward with a large portion free, diaphragm absent. Margin entire, circular, flaring slightly, often renovated, aperture diameter 334–418 μ.

Male and female gonothecae borne together in oval coppiniae consisting of elongate, curved tubes. Male gonothecae oval and pedicellate, female gonothecae sessile, compressed, truncate distally, with a narrow collar.

Remarks — Whereas Naumov (1960) reported this hydroid mainly from muddy bottoms, all the present records were from rocky substrates except station 65-1506, where the bottom type was unrecorded. Fertile colonies of this species were seen only from station 811 in northern Foxe Basin.

Distribution — Northern Canada, west, east Greenland, Iceland, Faeroes, Jan Mayen Island, Spitzbergen, Barents Sea, Kara Sea, Laptev Sea, East Siberian Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

Grammaria immersa Nutting, 1901
Plate V, Fig. 2

Collection records — Station 811.

Description — Fragments of colonies present only, reaching 3.5 cm long, consisting of an upright, polysiphonic rhizocaulus. Branches given off nearly perpendicular to the rhizocaulus, constricted basally but nearly the same diameter as the rhizocaulus elsewhere, polysiphonic throughout, terminating abruptly. Hydrothecae tubular, arranged in about 7–8 longitudinal series on rhizocaulus and branches, curving outward but with a very small portion free, diaphragm absent. Margin entire, circular, parallel or nearly so to the branch axis, not flaring, aperture diameter 167–230 μ.

Coppinia not seen.

Remarks — There is little in most descriptions of this species to distinguish it from *G. gracilis*, described by Stimpson (1854) from Grand Manan Island, N.B., and known only from the western Atlantic. Both exhibit similar

branching, are less robust than *G. abietina*, and have strongly curved and largely immersed hydrothecae. According to Fraser (1946) the hydrothecae of *G. gracilis* are smaller than those of *G. immersa*, but measurements were not given. Coppiniae are reported by Fraser (1937) as globular in *G. immersa*, elliptical in *G. gracilis* (Fraser, 1944). A taxonomic examination of these species is needed to determine whether or not they are conspecific.

Distribution — Northern Canada (new record), west, east Greenland, Iceland, Faeroes, Spitzbergen, Barents Sea, White Sea, Kara Sea, Laptev Sea, East Siberian Sea, Chukchi Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

Lafoea dumosa (Fleming, 1828)
Plate V, Fig. 3

Lafoea ramosa Packard, 1863, p. 404.

Lafoea dumosa Packard, 1867, p. 265; 1891, p. 369; Fraser, 1922, p. 5; 1931, p. 481; 1944, p. 221, fig. 205.

Collection records — Stations 33, 58.

Description — Colony creeping, or upright with a polysiphonic rhizocaulus reaching 1 cm high, pedicels arising from all sides of the rhizocaulus at an angle typically less than 45°. Hydrothecae tubular, curved, merging almost imperceptably with a short pedicel, pedicel slightly twisted occasionally, but not annulated. Hydrothecae 662–927 μ long, 252–291 μ wide, 2.4–3.3 times longer than wide, hydrothecal margin flaring very slightly, occasionally renovated.

Coppinia not seen.

Remarks — This species is readily distinguishable from *L. fruticosa* and *L. gracillima* by the short, smooth pedicel, making the hydrotheca appear almost sessile.

Distribution — Northern Canada, west Greenland, Iceland, Faeroes, Spitzbergen, Barents Sea, White Sea, Kara Sea, Laptev Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

Lafoea fruticosa M. Sars, 1851
Plate V, Fig. 4

Lafoea fruticosa Broch, 1907, p. 6, fig. 1; Fraser, 1922, p. 5; 1931, p. 481; Kramp, 1932b, p. 30; Fraser, 1944, p. 223, fig. 206.

Collection records — Stations 103, 319, 616, 65-1506. Also N.M.C. collection, mouth of Lake Harbour, Baffin Island, 55 m, August 4, 1939.

Description — Colony occasionally creeping but usually upright with a polysiphonic rhizocaulus reaching 2 cm high, branching irregular, pedicels arising from all sides of the rhizocaulus and branches at an angle typically more than 45°. Hydrothecae cylindrical, somewhat asymmetrical, abcauline wall convex, adcauline wall convex basally, concave distally, 662–861 μ long, 298–331 μ wide at the margin, 2.2–2.6 times longer than wide. Margin entire, slightly flaring, pedicels spirally coiled several times.

Coppinia not seen.

Remarks — The differences between robust colonies of *L. gracillima* and delicate colonies of *L. fruticosa* are relatively small, and the two species have been synonymized, most recently by Naumov (1960). However, Broch

(1918) showed that the abcauline surface of the hydrotheca in *L. gracillima* lacked the basal convexity present in morphologically similar colonies of *L. fruticosa*. The typical forms of these two species are quite dissimilar, particularly in the size and shape of the hydrotheca.

Distribution — Northern Canada, west, east Greenland, Iceland, Faeroes, Spitzbergen, Barents Sea, northern USSR (?), Sea of Japan, Bering Sea, Alaska.

Lafoea gracillima (Alder, 1856)
Plate V, Fig. 5

Lafoea gracillima Broch, 1907, p. 6, fig. 2; Fraser, 1922, p. 5; 1931, p. 481; 1944, p. 224, fig. 207.

Collection records — Stations 13, 28, 33, 58, 208, 210, 221, 225, 226, 331, 333, 334, 547, 616, 811, 820, 821, 59-21, 59-50, 59-66, 59-68, 60-1001, 61-1, 61-5, 61-15, 61-26, 62-2012, 62-4001, 62-4003, 63-020, 63-025, 64-004, 65-1506. Also N.M.C. collection, south of Bartlett Island, Hudson Bay, July 14, 1963, collector A. H. Clarke.

Description — Occasionally creeping but usually upright colonies with a polysiphonic rhizocaulus reaching 5 cm high, branching irregular, pedicels arising from all sides of the rhizocaulus and branches at an angle typically less than 45°. Hydrothecae tubular, curved, adcauline wall convex, at least basally, abcauline wall straight or concave, 530–761 μ long, 139–192 μ wide at the margin, 3.2–4.8 times longer than wide. Margin entire, slightly flaring, occasionally renovated, pedicels spirally coiled about 2–5 times, merging into the hydrotheca.

Coppinia not seen.

Distribution — Northern Canada, west, east Greenland, Iceland, Faeroes, Jan Mayen Island, Spitzbergen, Barents Sea, northern USSR, Bering Sea, Alaska.

Family SERTULARIIDAE
Abietinaria abietina (Linnaeus, 1758)
Plate V, Fig. 6

Sertularia abietina Packard, 1867, p. 265; 1891, p. 369; Whiteaves, 1901, p. 25.

Abietinaria abietina Kirchenpauer, 1884, p. 31; Fraser, 1944, p. 238, fig. 223.

Collection records — Stations 126, 616, 65-1502, 65-1506.

Description — Largest colony 4.7 cm high, hydrocaulus monosiphonic, geniculate, nodes oblique, regularly placed but often indistinct, each internode typically with a proximal apophysis and three alternating hydrothecae, the most proximal being axillary. Branches alternate, on two sides of the hydrocaulus, unbranched, divided into internodes of variable length. Hydrothecae large, smooth, alternate or subopposite, flask-shaped, wide basally, narrowing to a tubular neck distally, margin expanded, smooth, oblique to the main axis of the stem, adcauline wall half or more than half free, operculum of one adcauline valve.

Gonothecae small relative to the hydrothecae, oval, smooth or slightly wrinkled transversely, borne on short pedicels and arising near the bases of the hydrothecae, neck short, cylindrical, terminal aperture large, submarginal teeth present.

Distribution — Northern Canada, west, east Greenland, Iceland, Faeroes, Spitzbergen, Barents Sea, White Sea, Kara Sea, Laptev Sea, Chukchi Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

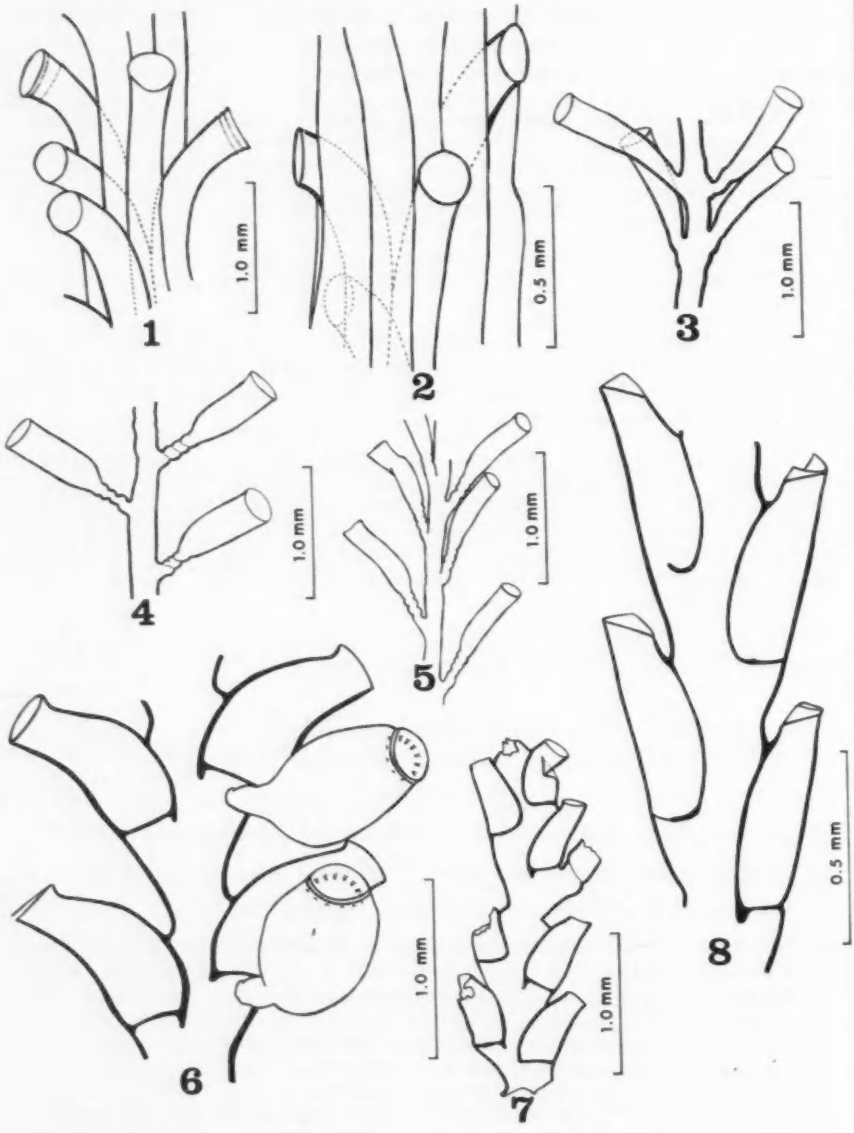

PLATE V. Fig. 1. *Grammaria abietina*, sta. 811; Fig. 2. *G. immersa*, sta. 811; Fig. 3. *Lafoea dumosa*, sta. 58; Fig. 4. *L. fruticosa*, sta. 65-1506; Fig. 5. *L. gracillima*, sta. 62-4003; Fig. 6. *Abietinaria abietina*, sta. 65-1506; Fig. 7. *A. turgida*, Ellef Ringnes Is.; Fig. 8. *Diphasia pulchra*, sta. 59-64.

Abietinaria turgida (Clark, 1876)
Plate V, Fig. 7

Abietinaria turgida Fraser, 1922, p. 5; 1931, p. 481; 1944, p. 240, fig. 225.

Collection records — N.M.C. collection, Isachsen, Ellef Ringnes Island, August 19, 1954, 46 m, collector S. D. MacDonald.

Description — Fragment of a branch only present, hydrothecae subopposite, closely placed, smooth, widest at the base, narrowing gradually to the margin, adcauline wall free a short distance, margin smooth, round, oblique to the main axis of the hydrotheca. Hydranths and opercular valves missing.

Gonothecae not seen.

Remarks — This identification was made on a single fragment of a colony in rather poor condition. The specimen bore some resemblance to descriptions of *A. annulata* (Kirchenpauer, 1884) and *A. gigantea* (Clark, 1876a), but the hydrothecae were more slender than in either of these species. The hydrothecal margin is nearly parallel to the main axis of the hydrocaulus in *A. gigantea* and perpendicular in *A. annulata*, whereas the margin was oblique in these specimens, as in *A. turgida*.

This is one of the few hydroid species from northern Canada that has not been reported in the north Atlantic.

Distribution — Northern Canada, East Siberian Sea, Chukchi Sea, Sea of Okhotsk, Bering Sea, Alaska.

Diphasia pulchra Nutting, 1904
Plate V, Fig. 8

Diphasia pulchra Fraser, 1922, p. 5; 1931, p. 481; 1944, p. 246, fig. 231.

Collection records — Station 59-64.

Description — Colony reaching 2.6 cm high, hydrocaulus monosiphonic, geniculate, divided at regular intervals by transverse nodes, each internode typically with a distinct apophysis proximally, and three hydrothecae, one axillary and two distally placed. Hydrocaulus twisted, branches arranged in a spiral distally, broken off proximally but apophyses spirally arranged, branches unbranched or dichotomously branched, divided into long, irregular internodes, each bearing several hydrothecae. Hydrothecae smooth, subalternate, nearly cylindrical, narrowest at the margin, widest in the midregion, pair widely separated front and back, abcauline wall 475–591 μ long, adcauline wall adnate for most of its length, free portion continuing upward, margin smooth with a large adcauline sinus, operculum of one adcauline valve.

Gonothecae not seen.

Remarks — Hydranths in the specimens from Hudson Bay were too decomposed to indicate the presence or absence of an abcauline caecum, but the species was placed in *Diphasia* rather than *Abietinaria* since Kramp (1932a) was unable to find an abcauline caecum in specimens from west Greenland.

Distribution — Northern Canada, west Greenland, Spitzbergen, Barents Sea, White Sea, Kara Sea, Laptev Sea, East Siberian Sea, Chukchi Sea, Sea of Okhotsk, Bering Sea.

Dynamena pumila (Linnaeus, 1758)
Plate VI, Fig. 1

Dynamena pumila Packard, 1863, p. 404; 1867, p. 265; 1891, p. 369.

Sertularia pumila Whiteaves, 1901, p. 25; Fraser, 1944, p. 286, fig. 274.

Collection records — N.M.C. collections, Bay of Exploits, Nfld., September 13, 1923, collector A. G. Huntsman; June 25, 1966, collectors D. E. McAllister, W. H. van Vliet.

Description — Colony reaching 1.7 cm high, hydrocaulus monosiphonic, proximal athecate portion twisted, branches irregularly placed when present, arising just below the hydrothecae on the proximal portion of the internode, smooth or twisted at their origin, occasionally rebranched. Hydrothecae opposite, two per internode, separated front and back, nearly cylindrical, curving outward with about $\frac{1}{3}$ of the adcauline wall free. Operculum of two valves, the abcauline larger, hydrothecal margin with two distinct lateral teeth and a small median adcauline tooth. Abcauline caecum absent.

Gonothecae arising below the hydrothecae from the proximal part of the internode, pear-shaped, often slightly rugose, terminating in a short collar, aperture wide.

Remarks — *Dynamena pumila* is a well-known Atlantic-boreal hydroid occurring primarily in the intertidal zone, although Naumov (1960) reported a specimen from a depth of 270 m. In Canadian waters it has been reported no further north than southern Labrador, and is unreported in the north Pacific except for a single questionable record by Clark (1876b) from California.

Distribution — Northern Canada, west Greenland, Iceland, Faeroes, Barents Sea, White Sea.

Sertularella pinnata Clark, 1876
Plate VI, Fig. 2

Sertularella pinnata Fraser, 1931, p. 481; 1944, p. 267, fig. 256.

Collection records — Station 616.

Description — A 7.5-mm long fragment of a branch present only, bearing 13 hydrothecae. Internodes 512–633 μ long, each with one hydrotheca, 111–163 μ wide at the node, nodes slightly or distinctly oblique. Hydrothecae alternate, short, tubular, pointed outward, total length of adcauline wall 453–517 μ, length free 238–348 μ, margin with three well-developed, pointed teeth, median adcauline tooth recurved toward the branch, width at margin 274–312 μ, perisarc thickened at the margin and at a point on the abcauline wall $\frac{1}{4}$–$\frac{2}{3}$ of the distance to the margin, opercular valves missing.

Gonothecae not seen.

Remarks — Originally described from Alaska by Clark (1876a), this is a distinct but evidently rare species, particularly in the Atlantic where it has been reported only from Jan Mayen Island, by Lorenz (1886).

Distribution — Northern Canada, Jan Mayen Island, Sea of Okhotsk, Bering Sea, Alaska.

Sertularella polyzonias (Linnaeus, 1758)
Plate VI, Fig. 3–5

Sertularia polyzonias Packard, 1863, p. 442; Whiteaves, 1901, p. 25.

Cotulina polyzonias Packard, 1867, p. 264; 1891, p. 369.

Sertularella polyzonias Fraser, 1922, p. 5; 1931, p. 481; 1944, p. 268, fig. 258.

Collection records — Stations 58, 109, 208, 210, 226, 616, 821, 59-21, 59-29, 59-50, 59-64, 61-1, 61-10, 65-1505, 65-1506.

Description — Colonies reaching 3.8 cm high, hydrocaulus monosiphonic, straight or slightly geniculate, unbranched or irregularly branched, branches usually long and like the hydrocaulus. Hydrocaulus and branches divided at regular intervals by oblique nodes sloping alternately in opposite directions, internodes varying from short to fairly long, widest at the base of the hydrothecae. Hydrothecae alternate, given off from the distal end of the internode, walls smooth, slightly wrinkled or distinctly corrugated, width maximal near the base, minimal just below the margin, distal portion quadrate, abcauline wall 1078–1400 μ long, length of adcauline wall free 866–1132 μ, adcauline wall adnate 493–612 μ, margin 453–559 μ wide, often renovated, with four equally developed teeth, operculum of four valves. Perisarc on hydrocaulus thin to thick, annulated at the base, wavy or annulated near the nodes.

Gonothecae oval, transversely furrowed, aperture distal, surrounded by four prominent spines.

Remarks — All the examined material from northern Canada corresponded to the robust form of this species, regarded by some authors (Nutting, 1904; Fraser, 1944; Naumov, 1960) as a distinct species, *S. gigantea* Mereschkowsky, 1878. Broch (1918), Vervoort (1946), and others have regarded *S. gigantea* as a synonym of *S. polyzonias* since Kirchenpauer (1884) reported intermediate forms between the typical and robust varieties. The robust form is more common in the Arctic than the typical form (Broch, 1918).

Distribution — Northern Canada, west, east Greenland, Iceland, Faeroes, Jan Mayen Island, Spitzbergen, Barents Sea, White Sea, Kara Sea, Laptev Sea, East Siberian Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

Sertularella tenella (Alder, 1856)
Plate VI, Fig. 6

Sertularella tenella Fraser, 1946, p. 77, 288.

Collection records — Station 59-66. Also N.M.C. collection, south of Bartlett Island, Hudson Bay, July 14, 1963, collector A. H. Clarke.

Description — Colonies reaching 1.1 cm high, hydrocaulus monosiphonic, geniculate, unbranched, divided at regular intervals by shallow oblique nodes sloping alternately in opposite directions, internodes 1.0–2.1 mm long, slender, 116–143 μ wide at the nodes, expanded distally. Hydrothecae alternate, widely separated, fusiform, widest in the midregion, narrowest just below the margin, borne distally on the internodes, abcauline wall 657–791 μ long, less than half of the adcauline wall adnate, length adnate 306–348 μ, length free 476–608 μ, walls rugose, distal portion of the hydrotheca quadrate, aperture perpendicular to the main hydrothecal axis, margin 253–306 μ wide, with four distinct, equally developed teeth. Perisarc on hydrocaulus thin to fairly thick, wrinkled near the nodes, annulated at the origin from the hydrorhiza but typically smooth elsewhere.

Gonothecae not seen.

Remarks — *Sertularella geniculata* Hincks, 1784, reported off the Newfoundland coast by Fraser (1944), is similar to and possibly synonymous with *S. tenella*. Hincks (1874) recognized the similarity of his species to *S. tenella*

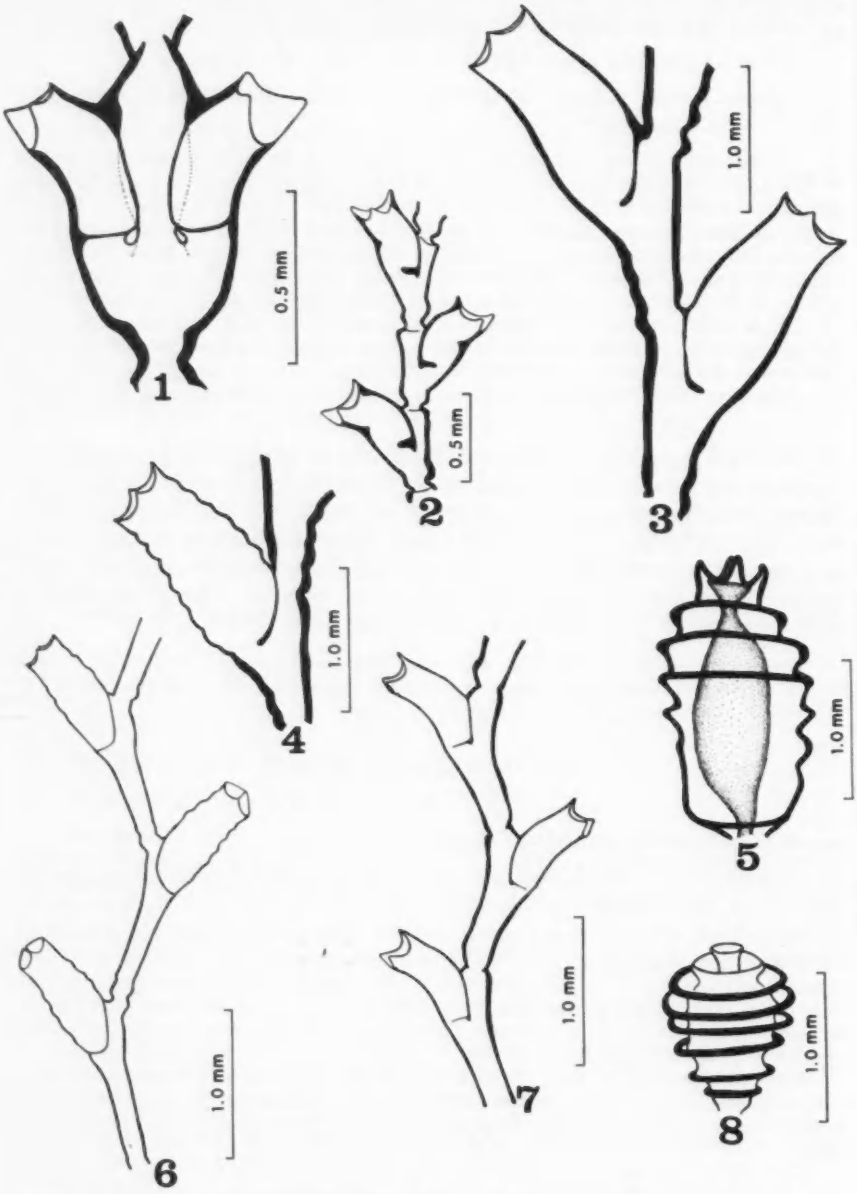

PLATE VI. Fig. 1. *Dynamena pumila*, Bay of Exploits, Nfld.; Fig. 2. *Sertularella pinnata*, sta. 616; Fig. 3. *S. polyzonias*, sta. 65-1505; Fig. 4. *S. polyzonias*, sta. 65-1506; Fig. 5. *S. polyzonias*, gonotheca, sta. 65-1505; Fig. 6. *S. tenella*, S of Bartlett Is., Hudson Bay; Fig. 7. *S. tricuspidata*, sta. 65-1506; Fig. 8. *S. tricuspidata*, gonotheca, sta. 65-1506.

but believed that it was distinct. The two species have since been regarded as synonymous by authors including Hartlaub (1901) and Broch (1910), whereas others, including Nutting (1904) and Fraser (1944), have recognized both. Specimens reported here more nearly correspond to descriptions of *S. tenella*.

Distribution — Northern Canada, west, east Greenland, Jan Mayen Island, Spitzbergen, Barents Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

Sertularella tricuspidata (Alder, 1856)
Plate VI, Fig. 7, 8

Sertularia tricuspidata Packard, 1863, p. 404; Whiteaves, 1901, p. 26.

Cotulina tricuspidata Packard, 1867, p. 264; 1891, p. 369.

Sertularella tricuspidata Kirchenpauer, 1884, p. 45; Broch, 1907, p. 8; Fraser, 1922, p. 5; 1931, p. 481; Kramp, 1932b, p. 42; Fraser, 1944, p. 274, fig. 264.

Collection records — Stations 33, 58, 106, 126, 201C, 203, 210, 225, 226, 319, 321, 547, 616, 59-21, 59-50, 59-64, 59-66, 60-1025, 61-1, 61-8, 61-26, 65-1044, 65-1506. Also N.M.C. collection, south of Bartlett Island, Hudson Bay, July 14, 1963, collector A. H. Clarke.

Description — Colonies reaching 2.5 cm high, hydrocaulus monosiphonic, geniculate, alternately or dichotomously branched, branches like the hydrocaulus, arising near the base of a hydrotheca, each axil thus being occupied by a single hydrotheca, branches usually long, given off at a wide angle and often perpendicular to the hydrocaulus, adding to the dichotomous appearance of the colony. Hydrocaulus and branches divided at regular intervals by shallow transverse or slightly oblique nodes, internodes 691–1200 μ long, slender, 111–180 μ wide at the nodes, each bearing a single hydrotheca near the distal end. Hydrothecae alternate, widely separated, cylindrical, curved outward, walls smooth, abcauline wall 492–565 μ long to tip of teeth, usually less than half of the adcauline wall adnate, length adnate 227–258 μ, length free 391–465 μ (to tip of adcauline tooth), margin 232–269 μ wide, with one adcauline and two abcauline teeth, all equally well developed, operculum of three valves, margin often renovated. Perisarc usually thin on hydrothecae and branches, thicker on the hydrocaulus, annulations present on the proximal-most branches and tendrils, usually smooth elsewhere except for a slight bulge near the nodes.

Gonothecae arising just below the hydrothecae on both hydrocaulus and branches, oval with deep transverse ribs, aperture distal, small, round, on a small tubuliform neck having a flaring margin.

Distribution — Northern Canada, west, east Greenland, Iceland, Faeroes, Jan Mayen Island, Spitzbergen, Barents Sea, White Sea, Kara Sea, Laptev Sea, East Siberian Sea, Chukchi Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

Sertularia cupressina Linnaeus, 1758
Plate VII, Fig. 1

Sertularia cupressina Packard, 1867, p. 265; 1891, p. 369.

Thuiaria cupressina Whiteaves, 1901, p. 27; Fraser, 1944, p. 298, fig. 283.

Collection records — Station 65-1505.

Description — Two fragments of a colony present, each about 1 cm long. Hydrocaulus monosiphonic, straight, divided into internodes of varying length, each internode with from 1

to 4 branch-bearing apophyses, each having an axillary hydrotheca, perisarc fairly thick, nodes occasionally indistinct. Apophyses given off more or less alternately from opposite sides of the hydrocaulus at an angle of less than 45° with the vertical. Branches having the broad surface horizontal, internodes of irregular length, usually short with 1 or 2 pairs of hydrothecae, branches dichotomously branched. Hydrothecae subopposite, closely placed, curving outward, the main axis oblique with that of the branch, abcauline wall 291–371 μ long, adcauline wall adnate 265–331 μ, over half its total length, margin with two distinct teeth, operculum with a small adcauline and a large, moveable abcauline valve, abcauline caecum present.

Gonothecae triangular, flattened, stout, arising near the bases of the hydrothecae, distal end with two rather short but distinct spines, orifice round, at the end of a short collar, submarginal teeth present.

Distribution – Northern Canada, Iceland, Barents Sea, White Sea.

Sertularia fabricii Levinsen, 1893
Plate VII, Fig. 2

Collection records — Station 65-1505.

Description — Species represented by a 2-cm long fragment, hydrocaulus monosiphonic, slightly geniculate, perisarc thick, internodes uniformly short, each with an apophysis proximally. Branches spirally arranged, dichotomously branched, forming a dense tuft distally, given off from the hydrocaulus at an angle of about 45°, broad surface horizontal, internodes of irregular length. Hydrothecae subalternate to subopposite, widest near the base, 139–205 μ in lateral view, tapering to the margin, curved slightly with the margin facing obliquely outward, abcauline wall 404–510 μ long, adcauline wall completely adnate or nearly so, length adnate 503–563 μ, distance between hydrothecae on same side 53–232 μ. Margin oval with two prominent teeth, operculum consisting of a small adcauline and a large moveable abcauline valve, abcauline caecum present.

Gonothecae not seen.

Distribution — Northern Canada (new record) west Greenland, Iceland, Jan Mayen Island, Kara Sea, Alaska.

Sertularia mirabilis (Verrill, 1872)
Plate VII, Fig. 3

Collection records – Stations 58, 229, 616, 61-1, 65-1505.

Description – Colonies reaching 3 cm high, hydrocaulus robust, monosiphonic, geniculate, branches arising alternately from opposite sides of the hydrocaulus, branches unbranched. Hydrothecae in two opposite rows on the hydrocaulus, six rows on the branches, sessile, between ⅓ and ½ of the adcauline wall free, widest at the base, tapering gradually to the margin, margin with two teeth, operculum of two valves, abcauline caecum present.

Gonothecae not seen.

Remarks — In Canadian waters this species has been reported northward to the Gulf of St. Lawrence and Grand Banks on the Atlantic coast, and to the Queen Charlotte Islands on the Pacific coast, but not from the Arctic.

Distribution — Northern Canada (new record), west Greenland, Iceland, Spitzbergen, Barents Sea, White Sea, Kara Sea, Sea of Japan, Bering Sea, Alaska.

Sertularia plumosa (Clark, 1876)
Plate VII, Fig. 4

Collection records – Station 61-26.

Description — Species represented by a 1.5-cm long fragment, hydrocaulus monosiphonic, geniculate, internodes uniform in length, fairly short, each giving off a branch proximally, perisarc thick. Each internode with three hydrothecae, one axillary and a pair distally. Upper side of the branch constricted at the origin, branches rigid, dichotomously branched, given off at an angle of about 45° or more with the vertical, arranged in a spiral about the hydrocaulus, broad surface horizontal, internodes of irregular length. Hydrothecae subopposite, closely placed, distance between two on same side 73–166 μ, adcauline wall entirely adnate or nearly so, length adnate 444–470 μ, abcauline wall 364–397 μ long, nearly parallel with the main axis of the branch, hydrothecae widest near the base, diameter 113–146 μ in lateral view, tapering gradually to the margin. Margin relatively small, with two distinct teeth, operculum consisting of a small adcauline and a large moveable abcauline valve, abcauline caecum present.

Gonothecae not seen.

Remarks — In general appearance this species resembles *S. robusta* and *S. fabricii* but is less robust and bushy in its colony form. *Sertularia plumosa* is also distinct in having smaller hydrothecae that are immersed to an extent nearly equal that seen in *Thuiaria laxa* and *T. thuja*.

Distribution — Northern Canada (new record), Barents Sea, White Sea, Kara Sea, Laptev Sea, East Siberian Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

Sertularia robusta (Clark, 1876)
Plate VII, Fig. 5, 6

Collection records — Stations 616, 61-43.

Description — Colonies reaching 5.5 cm high, hydrocaulus monosiphonic, geniculate, several very short internodes at the proximal end, elsewhere internodes moderately short, uniform in length, with one proximal and two distal hydrothecae and a branch given off just below the proximal hydrotheca. Branches distinctly constricted on the upper side at the origins, most broken off beyond the first internode, the remaining stubs given off at an angle of about 45° with the vertical and arranged in a spiral about the hydrocaulus. Well-developed branches absent on examined material, partially complete branches with the broad surface horizontal, divided at irregular intervals by nodes, evidently rebranched dichotomously. Hydrothecae subopposite, widest near the midregion, tapering distally, those located proximally on the branches having their main axis parallel or nearly so with that of the branch, a small portion of the abcauline wall free, margin not turned outward, those located distally on the branches having their main axis oblique to that of the branch, a relatively large portion of the adcauline wall free, and the margin turned outward, length of abcauline wall 457–500 μ. Margin oval with two distinct teeth, operculum consisting of a small adcauline and a large moveable abcauline valve, abcauline caecum present.

Gonothecae not seen.

Distribution — Northern Canada (new record), west Greenland, Barents Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

Sertularia schmidti Kudelin, 1914
Plate VII, Fig. 7–9

Collection records — Stations 33, 58, 126, 201C, 203, 206, 221, 226, 231, 321, 406, 547, 609, 616, 722, 811, 837, 59-29, 61-11, 61-26, 61-43, 65-1502, 65-1505.

Description — Colonies reaching 2.5 cm high, hydrocaulus monosiphonic, geniculate, inconspicuous, being nearly or equally as slender as the branches, divided at irregular intervals by transverse nodes. Internodes having a pair of subopposite hydrothecae only, a branch apophysis

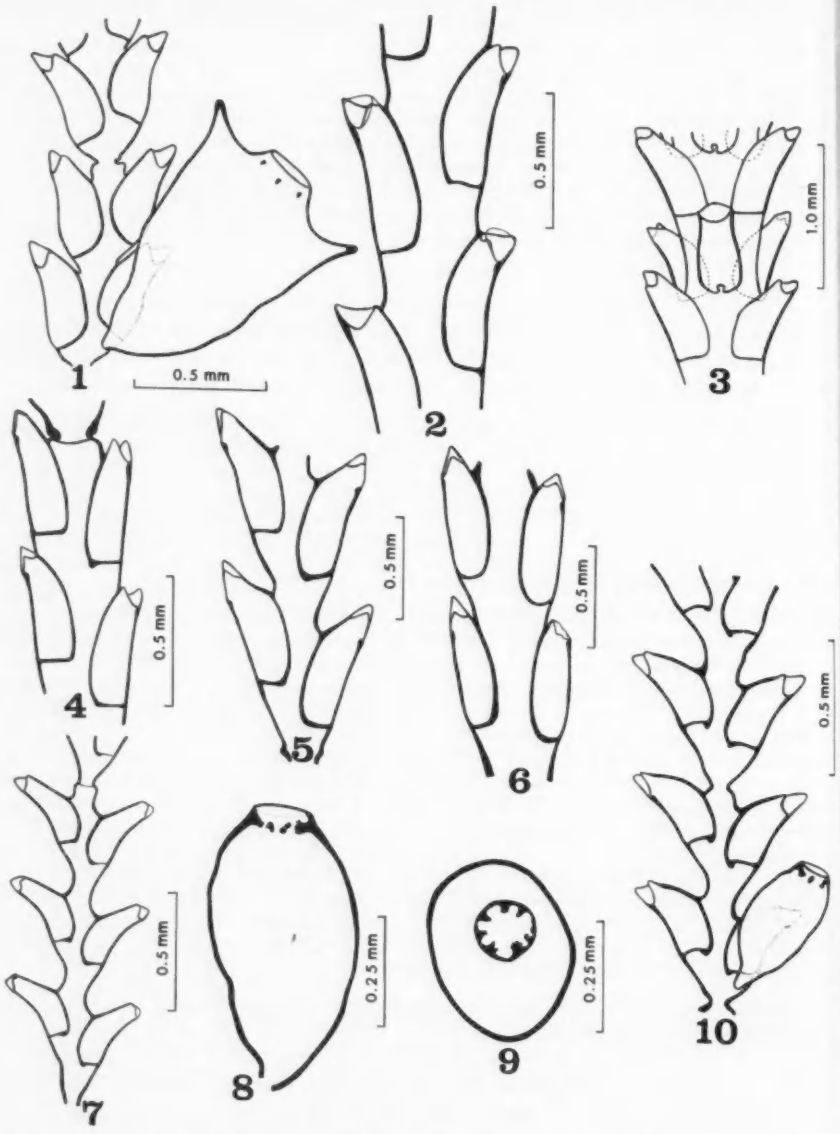

PLATE VII. Fig. 1. *Sertularia cupressina*, sta. 65-1505; Fig. 2. *S. fabricii*, sta. 65-1505; Fig. 3. *S. mirabilis*, sta. 65-1505; Fig. 4. *S. plumosa*, sta. 61-26; Fig. 5. *S. robusta*, hydrothecae on distal end of branch, sta. 616; Fig. 6. *S. robusta*, hydrothecae on proximal end of same branch, sta. 616; Fig. 7. *S. schmidti*, sta. 61-11; Fig. 8. *S. schmidti*, gonotheca, sta. 61-11; Fig. 9. *S. schmidti*, gonotheca, sta. 61-11; Fig. 10. *S. similis*, sta. 65-1505.

with an axillary hydrotheca only, or a proximal apophysis with an axillary hydrotheca and an additional one or two pairs of subopposite hydrothecae distally. Apophyses given off more or less alternately from opposite sides of the hydrocaulus at an angle usually somewhat more than 45° with the vertical. Branches with the broad surface vertical, internodes of irregular length, usually with 1–3 pairs of hydrothecae, branches unbranched or dichotomously branched. Hydrothecae small, subopposite, elongate, tapering distally, curved outward, the abcauline wall concave, 243–286 μ long, adcauline wall adnate 190–226 μ, free 167–210 μ, convex in the adnate portion, straight or nearly so along the free portion, opening small, margin with two teeth, operculum consisting of a small adcauline and a larger, moveable abcauline valve, abcauline caecum present.

Gonothecae obovate, about 570–650 μ long, irregularly oval in cross-section, arising near the bases of the hydrothecae, walls smooth, aperture terminal, circular, about 130 μ in diameter, neck low, indistinct, well-developed submarginal teeth present.

Remarks — This species, known elsewhere only from the White Sea near the Solovetsky Islands (Naumov, 1960), was common in the *Calanus* collections from the eastern Canadian Arctic. Of the hydroid species in arctic North America, *S. schmidti* could be most easily confused with *S. similis*, but is distinctly finer and has more slender hydrothecae with a relatively larger portion of the adcauline wall free. As Naumov noted, the hydrotheca shape is rather suggestive of that seen in certain species of *Abietinaria*. Present collections indicate that *S. schmidti* is widespread in the eastern Canadian Arctic, having been found in Foxe Basin, Hudson Bay, Hudson Strait, Ungava Bay, Frobisher Bay, and the Strait of Belle Isle. The gonothecae, described here for the first time, were found on colonies from stations 203, 206 (Ungava Bay), 226 (Hudson Strait), 61-11 (Hudson Bay) and 65-1505 (Strait of Belle Isle).

Distribution — Northern Canada (new record), White Sea.

Sertularia similis Clark, 1876
Plate VII, Fig. 10

Thuiaria similis Fraser, 1922, p. 5; 1931, p. 481; 1939, p. 59; 1944, p. 307, fig. 294.

Collection records — Stations 11, 33, 58, 203, 229, 318, 321, 334, 520, 547, 616, 811, 837, 901, 59-21, 59-29, 59-64, 61-10, 61-18, 61-24, 61-26, 61-43, 61-1015, 65-1044, 65-1505, 65-1506. Also N.M.C. collection, mouth of Lake Harbour, Baffin Island, 55 m, August 4, 1939.

Description — Colonies reaching about 3 cm high, hydrocaulus monosiphonic, straight or slightly geniculate, conspicuous, being more robust than the branches, divided at regular intervals by indistinct nodes into internodes having a proximal apophysis with an axillary hydrotheca, and two distally placed subopposite hydrothecae. Apophyses given off alternately from opposite sides of the hydrocaulus at an angle of about 45–90° with the vertical. Branches with the broad surface vertical, internodes of varying length, usually with 1–2 pairs of hydrothecae, branches unbranched or dichotomously branched. Hydrothecae subopposite, curved outward, abcauline wall concave, 206–266 μ long, adcauline wall convex, adnate for about half of its length, length adnate 196–283 μ, length free 157–233 μ. Margin with two distinct teeth, operculum consisting of a small adcauline and a larger, moveable abcauline valve, abcauline caecum present.

Gonothecae obovate, irregularly oval in cross-section, arising near the bases of the hydrothecae, walls smooth, aperture terminal, circular, on an indistinct neck, submarginal teeth present, well developed.

Remarks — This was one of the most common and widespread species in the examined collection, occurring from the Strait of Belle Isle westward

to Liverpool Bay, N.W.T. It has not been reported from Greenland, Iceland, or northern Europe, but is very common in the far eastern seas of the USSR (Naumov, 1960) and is abundant on the Pacific coast of North America (Fraser, 1946).

Distribution — Northern Canada, Sea of Okhotsk, Sea of Japan, Chukchi Sea, Bering Sea, Alaska.

Sertularia tenera G. O. Sars, 1874
Plate VIII, Fig. 1

Thuiaria tenera Fraser, 1939, p. 59.

Collection records — Stations 33, 58, 203, 811.

Description — Colonies reaching 6.7 cm high, hydrocaulus monosiphonic, divided at irregular intervals by nodes, distal $\frac{2}{3}$ or more of the hydrocaulus twisted, branches arranged spirally, arising from large apophyses, dichotomously branched, broad surface horizontal when spirally arranged, divided at frequent intervals by nodes, one to four hydrothecae per internode. Hydrothecae subalternate to alternate, widest in the midregion, tapering proximally and distally, diameter of base in lateral view 143–201 μ, about half the adcauline wall free, 232–306 μ free, 280–343 μ adnate, directed outward and frequently concave, abcauline wall nearly straight. Distance between hydrothecae on same side 459–780 μ. Margin oval with two distinct lateral teeth, operculum consisting of a small adcauline and a larger, moveable abcauline valve, abcauline caecum present.

Gonothecae oval, polygonal in cross-section, arising near the bases of the hydrothecae, truncate distally with a large aperture, submarginal teeth present.

Distribution — Northern Canada, west, east Greenland, Iceland, Faeroes, Spitzbergen, Barents Sea, White Sea, Kara Sea, Laptev Sea, East Siberian Sea, Chukchi Sea, Sea of Okhotsk, Sea of Japan, Bering Sea, Alaska.

Thuiaria alternitheca Levinsen, 1893
Plate VIII, Fig. 2

Collection records — Station 62-032. Also N.M.C. collection, *Loubyrne* Expedition station 19, 61°11′N, 90°00′W, Hudson Bay, August 15, 1930.

Description — Colonies reaching 11 cm high, hydrocaulus monosiphonic, up to 1.5 mm wide, nodes irregularly placed but distinct. Small colonies with branches arising from opposite sides of the hydrocaulus, larger colonies with the hydrocaulus twisted, branches given off from all sides on distinct apophyses. Branches long, unbranched or dichotomously branched, broad surface of the branches horizontal. Hydrothecae in two rows on the branches, smooth, alternate, immersed, crowded, adcauline wall entirely adnate, margins oval or nearly round and turned alternately from side to side, abcauline caecum present.

Gonothecae elongate, cone-shaped, arising near the bases of the hydrothecae, smooth, terminal aperture large, bounded by an indistinct collar, submarginal teeth present.

Remarks — Billard (1925) has been followed in recognizing *Thuiaria* as distinct from *Salacia*, the latter lacking the abcauline caecum present in the former.

Distribution — Northern Canada (new record), west Greenland, Iceland, Sea of Okhotsk, Alaska.

Thuiaria carica Levinsen, 1893
Plate VIII, Fig. 3

Collection records — Stations 226, 59-66.

Description — Species represented by fragments only, the largest 2.3 cm long. Hydrocaulus monosiphonic, divided at irregular intervals into long internodes, each with several alternating apophyses and subalternate hydrothecae, hydrothecae and apophyses on two sides of the hydrocaulus only. All branches broken off but one, branch unbranched, divided at irregular intervals by distinct nodes, hydrothecae subalternate, smooth, in lateral view 185–238 μ wide at the base, abcauline wall 343–454 μ long, length of adcauline wall adnate 353–513 μ, length free 185–285 μ, free portion turned outward, margin smooth, parallel or nearly so to the main axis of the branch, diameter 138–174 μ (adcauline wall to abcauline wall), operculum of one abcauline valve, abcauline caecum present.

Gonothecae not seen.

Remarks — This species was reported in North America from the Strait of Georgia (Fraser, 1918, 1937) and the Miramichi Estuary (Fraser, 1926, 1944). Descriptions and figures, given in all but the 1926 paper, were all obviously made from the same specimens. This is unfortunate because Fraser's specimens, if correctly identified, were atypical, having narrow rather than broad internodes, and alternate and widely separated rather than subalternate and rather closely placed hydrothecae.

Distribution — Northern Canada (new record), west Greenland, Iceland, Spitzbergen, Barents Sea, White Sea, Kara Sea, Laptev Sea, East Siberian Sea, Chukchi Sea, Sea of Okhotsk, Sea of Japan, Bering Sea.

Thuiaria laxa Allman, 1874
Plate VIII, Fig. 4

Thuiaria laxa Kramp, 1932b, p. 47.

Collection records — Stations 65-1502, 65-1506.

Description — Colony 1.5 cm high, hydrocaulus monosiphonic, geniculate, nodes absent, perisarc relatively thick, branches unbranched, not divided into internodes, curved upward slightly, broad surface vertical, arising alternately from opposite sides of, and at an angle with the hydrocaulus of more than 45° but less than 90°, apophyses short, 3–5 hydrothecae between successive apophyses on a given side. Hydrothecae in two rows on the branches, alternate or subalternate, adcauline wall completely adnate, 411–470 μ long, length of abcauline wall 316–359 μ, diameter at the base 147–174 μ in lateral view, distance between hydrothecae on same side 0–90 μ, margin nearly hemispherical, smooth, diameter 82–93 μ abcauline wall to adcauline wall, operculum of one abcauline valve.

Gonothecae not seen.

Remarks — The specimens referred to this species were small and identified with caution because Broch (1918) observed that small colonies of *T. thuja* are pinnate and easily confused with *T. laxa*. The identification as *T. laxa* was made because the hydrothecae at the tip of the branches lacked the two lateral teeth present in similarly situated hydrothecae of *T. thuja*, and the hydrothecae were smaller, more completely immersed and more crowded than those of *T. thuja*. The specimens most closely resembled Fraser's (1944)

description of *Thuiaria immersa* Nutting, 1904, a species synonymized with *T. laxa* by Broch (1910).

Distribution — Northern Canada, west, east Greenland, Iceland, Spitzbergen, Barents Sea, White Sea, Kara Sea, East Siberian Sea, Sea of Okhotsk, Sea of Japan.

Thuiaria lonchitis (Ellis and Solander, 1786)
Plate VIII, Fig. 5

Collection records — Station 59-69.

Description — Colony 4.1 cm high, hydrocaulus monosiphonic, geniculate, nodes distinct, irregularly placed. Branches unbranched, nearly straight, broad surface vertical, arising alternately from opposite sides of, and at an angle of about 45° with the hydrocaulus, apophyses short, three hydrothecae between successive apophyses on a given side. Hydrothecae in two rows on the branches, smooth, alternate, largely immersed, adcauline wall adnate 467–572 μ, free 26–132 μ, length of abcauline wall 397–434 μ, margin oval, smooth, diameter 150–180 μ (adcauline wall to abcauline wall), operculum of one abcauline valve, abcauline caecum present.

Gonothecae not seen.

Remarks — Fraser (1944) distinguished *T. kolaensis*, having a smooth hydrothecal margin, from *T. lonchitis*, having a toothed margin. Broch (1910, 1918) showed that the margin frequently appears to be toothed in *T. lonchitis* because of a more or less distinct abcauline sinus, and he regarded the two as conspecific.

Distribution — Northern Canada (new record), west Greenland, Iceland, Faeroes, Spitzbergen, Barents Sea, White Sea, Kara Sea, Laptev Sea, East Siberian Sea, Sea of Okhotsk, Sea of Japan, Bering Sea.

Thuiaria thuja (Linnaeus, 1758)
Plate VIII, Fig. 6

Thuiaria thuja Packard, 1863, p. 422; 1867, p. 264; 1891, p. 369; Fraser, 1933, p. 566; 1944, p. 309, fig. 297.

Collection records — Stations 334, 547, 815, 65-1506.

Description — Colonies reaching 14 cm high, hydrocaulus monosiphonic, nodes absent, branches broken off proximally, distally branches arising from distinct apophyses at regular intervals, and in a spiral from three sides of the hydrocaulus, giving the colony a bottle-brush appearance, branches short, stiff, dichotomously branched, curved, pointing upward distally but perpendicular or nearly so to the hydrocaulus near the origin. Hydrothecae in two rows on the branches, smooth, subalternate, immersed, adcauline wall 466–519 μ long, completely adnate, abcauline wall 354–412 μ long, margin 63–85 μ from abcauline wall to adcauline wall, nearly hemispherical, smooth except for the most distal hydrothecae on the branches, these having two rounded lateral teeth. Operculum of one abcauline valve, abcauline caecum present.

Gonothecae ovoid, arising near the bases of the hydrothecae, smooth or with faint transverse lines, terminating in a round aperture bounded by a short collar, submarginal teeth present.

Distribution — Northern Canada, west Greenland, Iceland, Faeroes, Barents Sea, White Sea, Chukchi Sea, Sea of Okhotsk, Bering Sea, Alaska.

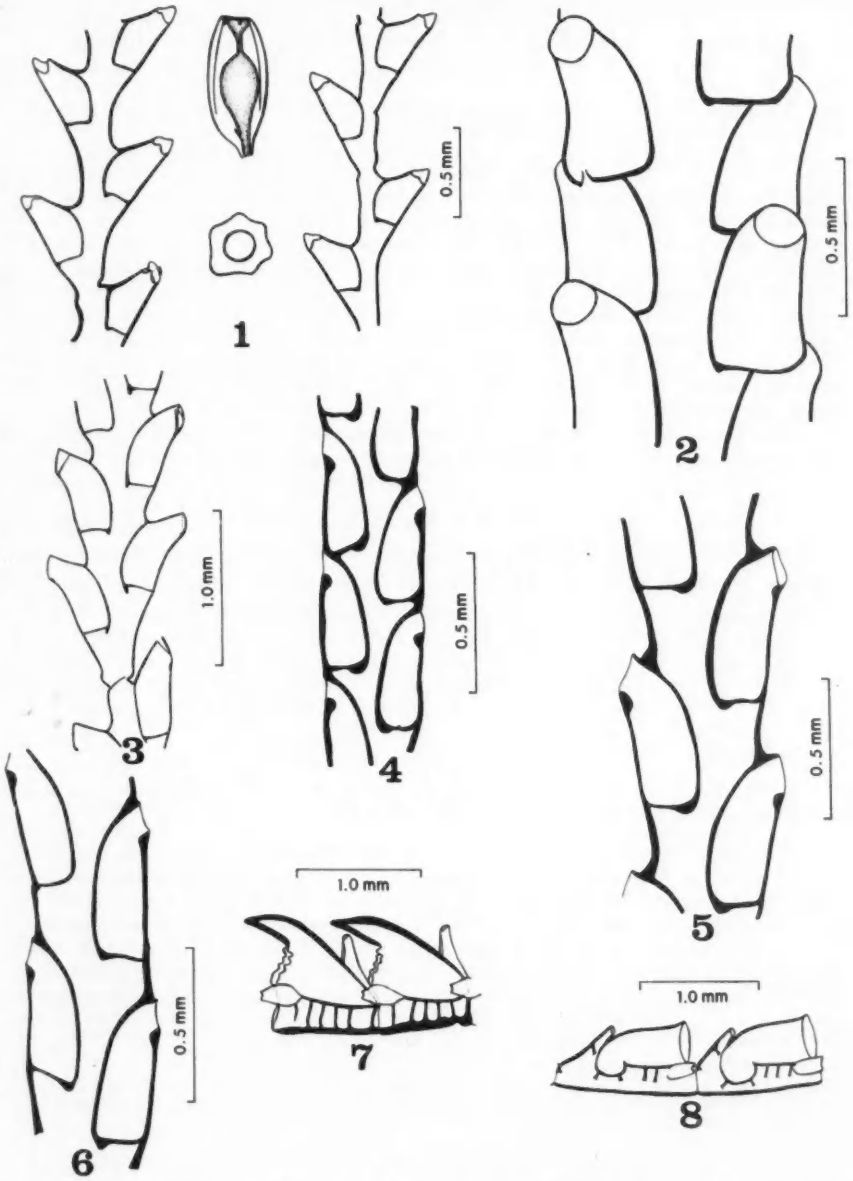

PLATE VIII. Fig. 1. *Sertularia tenera*, sta. 203; Fig. 2. *Thuiaria alternitheca*, *Loubyrne* sta. 19; Fig. 3. *T. carica*, sta. 226; Fig. 4. *T. laxa*, sta. 65-1502; Fig. 5. *T. lonchitis*, sta. 59-69; Fig. 6. *T. thuja*, sta. 65-1506; Fig. 7. *Cladocarpus cornutus*, NE Nfld.; Fig. 8. *C. integer*, NE Nfld.

Family PLUMULARIIDAE

Cladocarpus cornutus Verrill, 1879
Plate VIII, Fig. 7

Collection records — N.M.C. collection, 51°22′30″N, 50°31′30″W, off northeast Newfoundland, 457 m, May 28, 1963, collector S. W. Gorman.

Description — Hydrocaulus erect, polysiphonic, unbranched, reaching 5 cm high, hydrocladia given off alternately from a single tube of the hydrocaulus, bearing phylactogonia, otherwise unbranched. Hydrocladial internodes short, each bearing a median and a pair of lateral nematothecae, and a hydrotheca occupying its entire length, 6–7 septal ridges per internode, intrathecal ridge absent. Hydrothecae cone-shaped with a median abcladial keel extending considerably beyond the margin, margin with about four teeth on each side of the keel. Nematothecae tubular, one-chambered, immovable, median nematotheca long and adcladially split, arising at the hydrothecal base and passing obliquely upward, about half free, margin serrated. Lateral nematothecae large, split adcladially, extending obliquely outward, margin serrated, extending beyond the node and hydrothecal margin. Hydrocaulus with large nematothecae of similar morphology to those of the hydrocladia.

Gonothecae arising from the hydrocaulus, sac-shaped, wider than thick, folded distally, the opening facing basolaterally. Gonothecae protected by phylactogonia arising near the base of the hydrocladia, phylactogonium bearing 1–2 hydrothecae.

Remarks — This hydroid, originally described from Banquereau, off Nova Scotia, appears to be restricted to the western Atlantic. Its known longitudinal range is less than 40°, from 26°W to 64°W.

Distribution — Northern Canada (new record), west Greenland, Iceland.

Cladocarpus integer (G. O. Sars, 1874)
Plate VIII, Fig. 8

Cladocarpus pourtalesi Fraser, 1931, p. 481; 1944, p. 407, fig. 395.

Collection records — N.M.C. collection, 51°22′30″N, 50°31′30″W, off northeast Newfoundland, 457 m, May 28, 1963, collector S. W. Gorman.

Description — Hydrocaulus reaching 6.5 cm high, polysiphonic, unbranched, hydrocladia unbranched, given off alternately from a single tube of the hydrocaulus, internodes 918–1090 μ long, 234–284 μ wide at the nodes, each with one median and a pair of lateral nematothecae and one hydrotheca. Hydrothecae 635–735 μ long, opening diameter 334–418 μ, tubular, occupying most of the internode, margin entire, septal ridges 4–6 per internode, 2–3 between the lateral nematothecae and the intrathecal ridge, and 1–3 proximally from the ridge. Nematothecae tubular, adcladially split, one-chambered, immovable, median nematotheca short, arising from a projection, adnate to and curving over the hydrothecal base. Lateral nematothecae distally located on the internode, cauline nematothecae present.

Gonothecae not seen.

Distribution — Northern Canada, west Greenland, Iceland, Spitzbergen, Barents Sea.

ADDITIONAL RECORDS

The following species have been reported from the study area but were not found in the present collections. Records of species collected at depths exceeding 300 m have been excluded from this list.

Family HALECIIDAE

Halecium halecinum (Linnaeus, 1758). Labrador (Packard, 1867, p. 264; Whiteaves, 1901, p. 24; Fraser, 1944, p. 194).

INCERTAE SEDIS

Cuspidella grandis Hincks, 1868. Labrador (Whiteaves, 1901, p. 24), Hudson Bay (Fraser, 1922, p. 5).

Family PHIALELLIDAE

Opercularella producta (G. O. Sars, 1874). Ellesmere Island (Broch, 1907, p. 7).

Family CAMPANULARIIDAE

Campanularia amphora (Agassiz, 1862). Labrador (Packard, 1867, p. 265).

Clytia hemisphaerica (Linnaeus, 1767). Labrador (Nutting, 1915, p. 54; Fraser, 1944, p. 138). This species was referred to by Nutting (1915) and Fraser (1944) as *C. johnstoni*.

Hartlaubella gelatinosa (Pallas, 1766). Labrador (Packard, 1863, p. 404), Hudson Strait (Fraser, 1931, p. 480).

Obelia plicata Hincks, 1868. Hudson Bay (Fraser, 1922, p. 4.).

Family LAFOEIDAE

Cryptolaria borealis Levinsen, 1893. Labrador (Kramp, 1932b, p. 34).

Lafoea pocillum Hincks, 1868. Labrador (Hincks, 1868, p. 304), Hudson Bay (Fraser, 1931, p. 481), Hudson Strait (Fraser, 1931, p. 481). *Calycella syringa* with the operculum missing could easily be mistaken for this species. Broch (1918) regarded *L. pocillum* as a synonym of *L. fruticosa*, but Naumov (1960) recognized both species.

Family BONNEVIELLIDAE

Bonneviella gracilis Fraser, 1939. Dease Strait, N.W.T. (Fraser, 1939, p. 59). There is little in Fraser's description of this species to distinguish it from *Campanularia integra*.

Family SERTULARIIDAE

Abietinaria filicula (Ellis and Solander, 1786). Labrador (Packard, 1867, p. 264; Whiteaves, 1901, p. 25).

Diphasia rosacea (Linnaeus, 1758). Labrador, Strait of Belle Isle (Packard, 1863, p. 404).

Sertularella fusiformis (Hincks, 1861). Hudson Strait (Fraser, 1922, p. 5).

Sertularella rugosa (Linnaeus, 1758). Labrador (Packard, 1867, p. 264; Kirchenpauer, 1884, p. 42.)

Sertularia argentea Linnaeus, 1758. Labrador (Packard, 1863, p. 422), Cumberland Sound (Verrill, 1879, p. 152). Hancock et al. (1956) have been followed in regarding *S. argentea* as a distinct species.

Thuiaria hippuris Allman, 1874. Labrador (Kramp, 1932b, p. 47).

Family PLUMULARIIDAE

Nemertesia antennina (Linnaeus, 1758). Labrador (Kramp, 1932b, p. 56).

ZOOGEOGRAPHY

Owing to the complex hydrography of the Arctic Ocean, arctic and subarctic zones require specific definition. Dunbar (1951) used the term "arctic" for shelf areas covered by polar water only, and "subarctic" for shelf areas covered by both polar and nonpolar water. On this basis, eastern Canada from Newfoundland northward to the Cumberland Peninsula, and westward through Hudson Strait to Foxe Peninsula, was considered subarctic. Dunbar also regarded the coastal region in northwestern Canada from Dolphin and Union Strait westward as subarctic.

As with the Polychaeta (Grainger, 1954) and Bryozoa (Powell, 1968), the thecate hydroid fauna of northern Canada consists largely of widely distributed species (Table 3). Of the 71 species recorded, 52 are known to transgress both arctic and subarctic zones, corresponding to Ekman's (1953) panarctic category. Of the exceptions, 10 species occur in the north Pacific as well as the boreal and subarctic zones of the Atlantic: *Halecium halecinum*, *Clytia hemisphaerica*, *Hartlaubella gelatinosa*, *Obelia plicata*, *Abietinaria filicula*, *Sertularella fusiformis*, *S. rugosa*, *Sertularia argentea*, *Thuiaria hippuris*, and *Nemertesia antennina*. Seven species, *Campanularia amphora*, *Gonothyraea loveni*, *Diphasia rosacea*, *Dynamena pumila*, *Sertularia cupressina*, *Cladocarpus cornutus* and *C. integer*, are Atlantic-boreal species capable of penetrating the subarctic but not the arctic. *Calycella gracilis*, known only from Helgoland and Ungava Bay, and *Bonneviella gracilis*, known only from Dease Strait, are too poorly known to categorize. There were no purely arctic species represented in the collections.

Of the 52 species transgressing both arctic and subarctic zones, 35 occur in the north Atlantic and north Pacific as well as the Arctic. Four species, *Sertularia fabricii*, *S. mirabilis*, *S. robusta*, and *Thuiaria thuja*, also occur in the North Atlantic and North Pacific but do not penetrate very far into arctic waters. *Cuspidella procumbens*, *Halecium curvicaule*, and *Cryptolaria borealis* correspond to Ekman's (1953) Atlantic–Arctic group, ranging eastward from northern Canada no farther than the Kara or Laptev seas. *Abietinaria turgida* and *Sertularia similis* appear to be of Pacific origin, not having been reported eastward from northern Canada or west of the East Siberian Sea. *Halecium speciosum* and *Sertularella pinnata* have each been reported once in the north Atlantic and are probably also of Pacific origin. Three species, *Cuspidella humilis*, *Obelia geniculata*, and *Filellum serpens*, are cosmopolitan, ranging from the arctic to the tropics. The remaining three species, *Halecium undulatum*, *Ptychogena lactea*, and *Sertularia schmidti*, are too poorly known to be categorized.

There is no significant barrier to the dispersal of animals westward through Baffin Bay and Davis Strait, and the similarity of the Canadian Arctic hydroid fauna to that of west Greenland is apparent (Table 3). Many of the thecate species in northern Canada also occur in the Alaska–Bering Sea region, but this area is rich in number of species and only about one-third of the thecate species reported by Fraser (1937) from Alaska occur in northern Canada. This may be

TABLE 3. Distribution of the thecate hydroids of northern Canada in other cold-water regions of the northern hemisphere.

Species	W Greenland	E Greenland	Iceland	Faeroes	Spitzbergen	Barents Sea	White Sea	Kara Sea	Laptev Sea	East Siberian Sea	Sea of Okhotsk	Sea of Japan	Chukchi Sea	Bering Sea–Alaska
Halecium curvicaule	+	+	+		+	+	+	+	+					
H. groenlandicum	+	+				+	+	+	+		+			+
H. halecinum		+	+	+	+	+					+	+		+
H. labrosum	+	+	+	+	+	+	+	+			+		+	+
H. minutum	+	+	+		+	+	+	+						+
H. muricatum	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H. scutum	+		+	+	+	+	+	+	+		+	+	+	
H. speciosum					+						+			+
H. undulatum	+		+	+										
Ptychogena lactea						+		+						
Cuspidella grandis	+	+				+		+						
C. humilis	+	+	+	+	+					+				
C. procumbens	+	+												
Opercularella lacerata	+		+	+		+	+							
O. producta														
Calycella gracilis														
C. syringa	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lafoeina maxima	+	+	+		+	+		+	+	+	+	+	+	+
Stegopoma plicatile	+	+	+		+	+	+	+	+		+	+		+
Tetrapoma quadridentatum	+	+			+	+	+	+		+	+			
Campanularia amphora														
C. groenlandica	+	+	+	+	+	+	+	+	+		+	+		+
C. integra	+	+	+	+	+	+	+	+	+		+	+	+	+
C. speciosa	+				+	+		+	+		+	+		+
C. verticillata	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C. volubilis	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Clytia hemisphaerica			+	+	+									+
Gonothyraea loveni	+		+	+	+	+	+							
Hartlaubella gelatinosa														
Obelia geniculata	+		+	+		+	+							
O. longissima	+	+	+	+	+	+	+	+	+	+	+	+	+	+
O. plicata														+
Cryptolaria borealis	+		+			+		+						
Filellum serpens	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grammaria abietina	+	+	+	+	+	+		+	+	+	+	+		+
G. immersa	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lafoea dumosa	+		+	+	+	+	+	+	+		+	+		+
L. fruticosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L. gracillima	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L. pocillum	+					+	+	+						+
Bonneviella gracilis														
Abietinaria abietina	+	+	+	+	+	+	+	+	+		+	+	+	+

(*Continued*)

TABLE 3. Distribution of the thecate hydroids of northern Canada in other cold-water regions of the northern hemisphere.—(*Concluded*)

Species	W Greenland	E Greenland	Iceland	Faeroes	Spitzbergen	Barents Sea	White Sea	Kara Sea	Laptev Sea	East Siberian Sea	Sea of Okhotsk	Sea of Japan	Chukchi Sea	Bering Sea–Alaska
A. filicula	+		+	+		+	+				+	+		+
A. turgida										+	+		+	+
Diphasia pulchra	+				+	+	+	+	+	+	+		+	+
D. rosacea			+	+		+	+							
Dynamena pumila	+		+	+		+	+							
Sertularella fusiformis					+									
S. pinnata											+			+
S. polyzonias	+	+	+	+	+	+	+	+	+	+	+	+		+
S. rugosa	+		+	+		+	+				+	+		+
S. tenella	+	+			+	+					+	+		+
S. tricuspidata	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sertularia argentea			+	+		+	+							+
S. cupressina			+	+		+	+							
S. fabricii	+		+					+						+
S. mirabilis	+		+		+	+	+	+				+		+
S. plumosa						+	+	+	+	+	+	+		+
S. robusta	+					+					+	+		+
S. schmidti							+							
S. similis											+	+	+	+
S. tenera	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thuiaria alternitheca	+		+								+			+
T. carica			+		+	+	+	+	+	+	+	+	+	+
T. hippuris	+													
T. laxa	+	+	+		+	+	+	+		+	+	+		
T. lonchitis	+		+	+	+	+	+	+	+	+	+	+		+
T. thuja	+		+	+		+	+				+		+	+
Nemertesia antennina	+		+	+	+	+								
Cladocarpus cornutus	+		+											
C. integer	+		+		+	+								
Total	51	29	47	35	39	51	41	37	27	22	40	32	21	44

related to the relatively minor contribution of Pacific water to the Canadian arctic. Although the Pacific influence is strong in the Chukchi Sea, it is weak in the northern Canadian islands and even in the southeast Beaufort Sea (Grainger, 1965).

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Vertebral and Other Meristic Characteristics of Greenland Halibut, *Reinhardtius hippoglossoides*, from the Northwest Atlantic

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TEMPLEMAN, W. 1970. Vertebral and other meristic characteristics of Greenland halibut, *Reinhardtius hippoglossoides*, from the northwest Atlantic. J. Fish. Res. Bd. Canada 27: 1549–1562.

Vertebral averages were not useful in separating Greenland halibut stocks of the northwest Atlantic, apart from the possible separation of the Gulf of St. Lawrence population.

Mean numbers of vertebrae did not differ significantly at the 5% level within the main areas in samples collected between 1950 and 1968 from Baffin Bay to the southern Grand Bank. Mean numbers of vertebrae for each of these main areas also were, with one exception, not significantly different. Mean vertebral numbers of Greenland halibut from the northern Gulf of St. Lawrence (61.850 ± 0.057 SE) differed ($P < 0.001$) from the overall mean for the remainder of the northwest Atlantic (61.575 ± 0.014).

The similar means were associated with similar temperatures at depths of 800 m and over, where eggs and early larvae are presumed to develop. Means of vertebral numbers of males and females were not significantly different.

Precaudal and caudal vertebral numbers were negatively correlated. Fused vertebrae were not numerous and did not show much localization except toward the posterior end of the vertebral column, especially in the two most posterior whole vertebrae. The numerical value of each fused partial vertebra was very close to one.

Anal and dorsal fin-ray numbers were highly correlated but were not correlated significantly with vertebral numbers. The means of dorsal and anal fin-ray numbers in a few samples from west Greenland to the southern Grand Bank were not significantly different.

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INTRODUCTION

MERISTIC CHARACTERISTICS, especially vertebral numbers, of Greenland halibut, *Reinhardtius hippoglossoides* (Walbaum, 1792) (recent official trade name in the United States and Canada, "Greenland turbot"), were studied on samples of this fish obtained from west Greenland and Baffin Island to the southern Grand Bank and the Gulf of St. Lawrence in 1950–68. The main purpose of this study was to discover whether these characteristics could be used in stock separation.

MATERIALS AND METHODS

The Greenland halibut were measured either at sea, or on shore after icing on the vessel, and usually were sexed. All whole vertebrae were counted and the urostylar half vertebra was counted as one vertebra. The first caudal vertebra was taken as the most anterior vertebra bearing

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a complete haemal arch with a definite haemal spine (Hubbs and Lagler, 1958). Vertebral columns with fused vertebrae were noted so that the effects of their exclusion from the means, which were of numbers of vertebrae in normal vertebral columns only, could be studied. Most vertebral columns were prepared for counting by cooking the trimmed fresh fish, removing the flesh by a water hose, and then drying the vertebral column. A smaller number was counted from radiographs. Much of the counting of dorsal and anal fin rays was done on research vessels, all rays being counted. Fin-ray counts were made on a smaller number of random samples, which were also used for vertebral counts, but occasional fish had imperfect or injured fins, and fin rays were not counted whereas others whose fin rays were counted had skeletons broken and parts missing so that no vertebral counts were obtained.

The fish used were random samples, usually from otter-trawl catches, except that some fish less than 30 cm in length were excluded because of probable breakage during cooking and cleaning. Because, as will be shown later, vertebral number in three separate periods of years was unrelated to fish length, the effects of this exclusion were probably not significant.

For additional tabular data related to this paper, including frequencies of meristic characteristics, see Templeman (MS, 1970).

NUMBERS OF VERTEBRAE

Apparently, with the possible exception of the northern Gulf of St. Lawrence population, vertebral averages, at least in the numbers and detail described in this paper, offer no aid in separating the Greenland halibut stocks of the northwest Atlantic.

Vertebral samples of male Greenland halibut from the same general areas and from the same or different years (Fig. 1; Table 1, 1–8, 10) were compared by analysis of variance and calculation of F values within each area and no significant differences were found. For the samples of females included in groups 1–8 and 10 (Table 1), there were no significant differences except in 3 ($P = 0.025$). Also, for the vertebral samples of male + female + unsexed included in these groups, there were no significant differences within each group. Hence the individual smaller samples indicated by means in Fig. 1 and two very small samples for which means were not calculated were combined by area and period into groups 1–8 and 10.

Furthermore, when these grouped vertebral frequencies from west Greenland and Baffin Island to the southern Grand Bank (Table 1, 1–8) were compared by analysis of variance for males, females, and totals of male + female + unsexed, separately, there were no significant differences between the frequencies. However, when sample 10 from the Gulf of St. Lawrence was included the samples differed ($P < 0.001$).

In these grouped samples there was no significant difference between vertebral means of males and females (t-test).

Vertebral means for samples of 15 or more fish obtained from the whole area from west Greenland and Baffin Island to the southern Grand Bank (not including the Gulf of St. Lawrence) ranged from 61.344 to 61.733 (Fig. 1), and the averages for the various areas (Table 1, 1–8) ranged from 61.515 to 61.627.

The mean (61.424) for a sample of 33 females from the deep water off the slopes of Flemish Cap and the eastern Grand Bank (Table 1, 9) differed

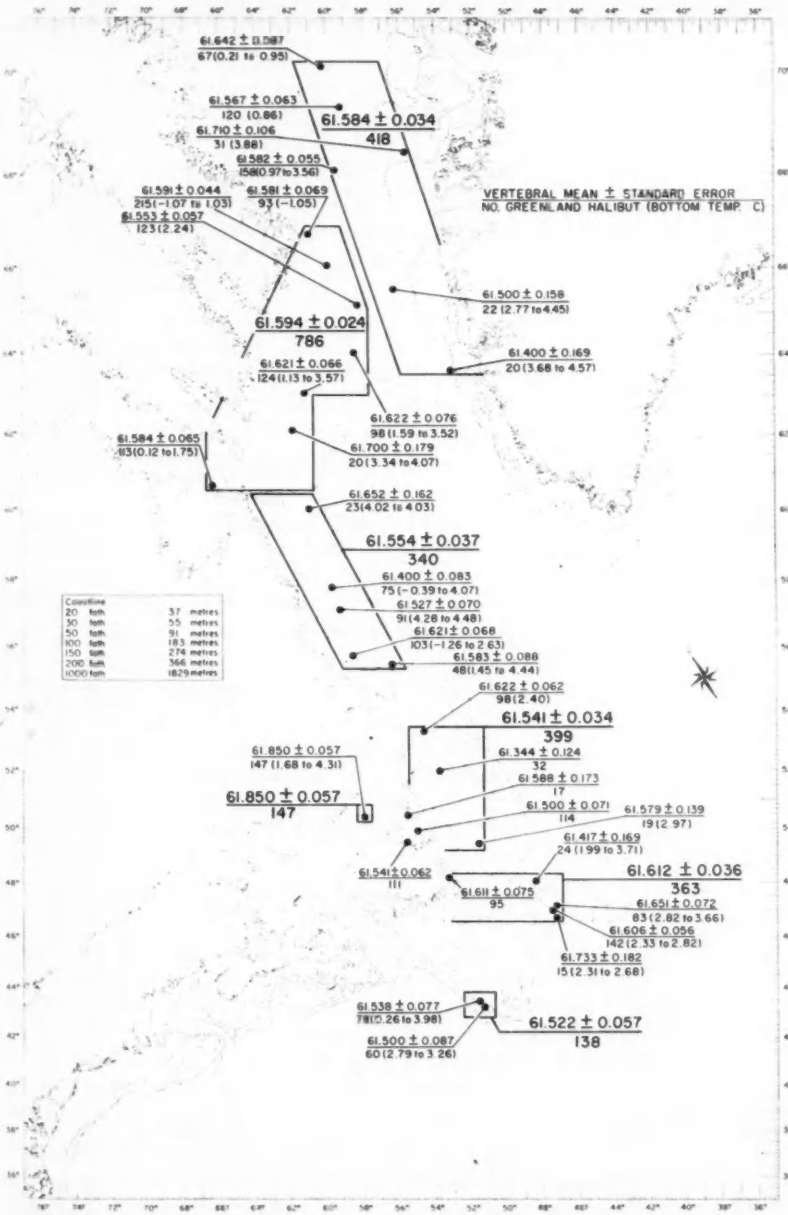

FIG. 1. Vertebral means of Greenland halibut from the northwest Atlantic, 1950–66. The smaller numerals are for individual samples at the points indicated and the larger ones are for the combinations of these samples within the areas outlined in this figure and in Table 1. (The values in each set of data are, in order: mean, one standard error of mean, number of fish, and bottom temperature (C). Two small samples are included in the large area samples but are not shown individually in the figure: eight fish included in the total for the NE Newfoundland Shelf and four fish from Flemish Cap included in the total for Grand Bank to SE Newfoundland coast. The sample of 33 females from the Flemish Cap–Grand Bank deep water, 1968, Table 1, no. 9, is not included in the figure.)

TABLE 1. Vertebral numbers of Greenland halibut from various areas of the northwest Atlantic, 195[illegible]. See Fig. 1 for distributions and sizes of the original samples.

	Area	Year	Depth (*m*)	Bottom temp (*C*)	Total no. fish	Sex	No. vertebrae Mean±SE[a]	Ra[illegible]
1.	W Greenland shelf 70°10′15″–63°38′N 53°00′–60°57′W	1965	366–1460	0.10–4.57	198	Male	61.596±0.049	60-[illegible]
					219	Female	61.575±0.047	60-[illegible]
					1	Unsexed	–	[illegible]
					418	Total	61.584±0.034	60-[illegible]
2.	Baffin Island shelf and Ungava Bay 66°42′–60°36′N 58°10′–66°21′W	1959	273–768	−1.05 to 4.07	490	Male	61.588±0.032	60-[illegible]
					296	Female	61.605±0.038	60-[illegible]
					786	Total	61.594±0.024	60-[illegible]
3.	Labrador shelf 60°00′–55°26′45″N 56°23′–60°50′W	1959, 1960	179–768	−1.26 to 4.48	166	Male	61.554±0.051	60-[illegible]
					174	Female	61.534±0.054	60-[illegible]
					340	Total	61.544±0.037	60-[illegible]
4.	NE Nfld. shelf 53°11′–49°25′N 51°30′–55°39′W	1950, 1951	274–375	0.12–2.97	34	Male	61.529±0.097	60-[illegible]
					40	Female	61.425±0.118	60-[illegible]
					227	Unsexed	61.529±0.047	60-[illegible]
					301	Total	61.515±0.040	60-[illegible]
5.	NE Nfld. shelf 53°14′N 54°35′W	1963	358–379	2.40	98	Total	61.622±0.062	60-[illegible]
4 & 5.	NE Nfld. shelf 53°14′–49°25′N 51°30′–55°39′W	1950–63	274–379	0.12–2.97	399	Total	61.541±0.034	60-[illegible]
6.	Grand Bank to SE Nfld. coast 48°08′–46°30′N 47°02′–53°13′W	1950, 1953	256–366	0.33–2.68	52	Male	61.538±0.101	59-[illegible]
					58	Female	61.707±0.095	61-[illegible]
					110	Total	61.627±0.069	59-[illegible]
7.	Grand Bank 48°04′–46°49′N 47°00′–48°57′W	1961	223–722	1.99–371	106	Male	61.594±0.063	60-[illegible]
					147	Female	61.612±0.057	59-[illegible]
					253	Total	61.605±0.042	59-[illegible]
6 & 7.	Grand Bank to SE Nfld. coast 48°08′–46°30′N 47°00′–53°13′W	1950–61	223–722	0.33–3.71	363	Total	61.612±0.036	59-[illegible]
8.	SW Grand Bank 43°51′15″–42°50′30″N 50°40′–52°19′W	1965–66	64–300	0.26–3.98	49	Male	61.653±0.095	61-[illegible]
					88	Female	61.455±0.072	60-[illegible]
					1	Unsexed	–	61
					138	Total	61.522±0.057	60-[illegible]
9.	Flemish Cap and E and SW Grand Bank 48°58′–43°42′N 43°45′–53°17′W	1968	1370–1630	3.62–3.98	33	All females	61.424±0.115	60-[illegible]
10.	Gulf of St. Lawrence 50°29′–50°17′15″N 57°38′30″–58°14′30″W	1965	179–322	1.68–4.31	62	Male	61.855±0.076	61-[illegible]
					83	Female	61.855±0.084	60-[illegible]
					2	Unsexed	–	61-[illegible]
					147	Total	61.850±0.057	60-[illegible]
11.	Baffin Island to SW Grand Bank (2–8) 66°42′–42°50′30″N 47°00′–66°21′W	1950–66	64–768	−1.26 to 4.48	897	Male	61.581±0.023	59-[illegible]
					803	Female	61.573±0.024	59-[illegible]
					326	Unsexed	61.555±0.038	60-[illegible]
					2026	Total	61.574±0.015	59-[illegible]
12.	W Greenland and Baffin Island to SW Grand Bank (1–8) 70°10′15″–42°50′30″N 47°00′–66°21′W	1950–66	64–783	−1.26 to 4.57	1095	Male	61.584±0.021	59-[illegible]
					1022	Female	61.573±0.022	59-[illegible]
					327	Unsexed	61.554±0.038	60-[illegible]
					2444	Total	61.575±0.014	59-[illegible]

[a]SE = standard error.

from the mean (61.855) for females from the Gulf of St. Lawrence ($P < 0.005$) but not significantly from the means for females from other areas.

The vertebral mean for the northern Gulf of St. Lawrence (61.850) differed from the overall mean for the remainder of the northwest Atlantic (Table 1, 12; 61.575; $P < 0.001$).

In the grouped samples (Table 1, 11) from the whole Canadian area (apart from the Gulf of St. Lawrence where means were equal for male and female), and from the whole Canadian area (apart from the Gulf of St. Lawrence) + west Greenland (Table 1, 12) the means for males and females did not differ significantly.

The mean for west Greenland (Table 1, 61.584 ± 0.034 SE) is higher ($P < 0.05$) than that (61.432 ± 0.062) of Hubbs and Wilimovsky (1964) for 88 Greenland halibut 76–103 mm in standard length taken at 69°30′N, 56°32′W, 202 m, west of Disko Island on June 27, 1925 (Dr J. Neilsen, Universitetets Zoologiske Museum, Copenhagen, personal communication). Some of the Greenland halibut of the 1965 sample were taken from the same area.

The mean for Baffin Island to SW Grand Bank (Table 1, 61.574 ± 0.015) is not significantly higher than that (61.273 ± 0.195) of Hubbs and Wilimovsky (1964) for 11 specimens of Greenland halibut taken in 1879–1963 from Labrador and the Grand Bank. This sample is, however, too small and too widely scattered in time and space to offer more than an indication of the correct average for the area.

The means, however, for the northwest Atlantic (Table 1) are about one vertebra lower than and different ($P < 0.001$) from the means given by Hubbs and Wilimovsky for Greenland halibut vertebral frequencies from the north Pacific. The vertebral numbers of Mikawa (1963) for Greenland halibut of the north Pacific are essentially similar to those of Hubbs and Wilimovsky.

Relation of Vertebral Number to Age or Period

Because vertebral average in fishes can vary with year-class and because age was not available, vertebral number was compared at different total length ranges of Greenland halibut and in three periods, 1950–53, 1959–63, and 1965–68 (Table 2). Because no difference in vertebral frequencies was found throughout the area except for the Gulf of St. Lawrence, all samples except those from the Gulf were used for this comparison.

Analysis of variance and calculation of F values showed no significant difference between the frequencies of vertebral number at the various length groups in each of the above periods nor between the total vertebral frequencies of all length groups for each of the three periods. Consequently there is little likelihood of significant bias in vertebral number due to year-class differences within the period 1950–68 during which these data were gathered.

TABLE 2. Vertebral numbers of Greenland halibut from the northwest Atlantic in relation to fish length and period.

1950–53 (Hawke Channel, Labrador, to E slope Grand Bank and the Newfoundland bays)						
Length range (*cm*)	20–39	40–49	50–89	–	–	20–89
No. vertebral columns	185	129	100	–	–	414
Mean vertebral no.	61.519	61.504	61.640	–	–	61.543
SE[a]	0.053	0.062	0.067	–	–	0.035
1959–63 (Baffin Island shelf, N Labrador shelf, and E slope Grand Bank)						
Length range (*cm*)	10–39	40–49	50–59	60–69	70–119	10–119
No. vertebral columns	134	308	503	315	119	1379
Mean vertebral no.	61.515	61.607	61.579	61.587	61.605	61.583
SE	0.062	0.036	0.031	0.038	0.065	0.018
1965–68 (W Greenland and E and SW slopes of Grand Bank)						
Length range (*cm*)	10–39	40–49	50–59	60–109	–	10–109
No. vertebral columns	144	145	172	105	–	566
Mean vertebral no.	61.569	61.566	61.593	61.505	–	61.564
SE	0.055	0.058	0.052	0.070	–	0.029

[a]SE = standard error.

PRECAUDAL AND CAUDAL VERTEBRAE

The numbers of precaudal and caudal vertebrae for the six west Greenland samples taken in 1965 were compared by analysis of variance and calculation of F values. The means of the various samples were not significantly different, and the samples were combined. The means for the west Greenland sample — precaudal, 17.666 ± 0.028, and caudal 43.922 ± 0.042 (293 fish, range 17–19 and 42–46 vertebrae) — were compared with those of a sample from the northern Gulf of St. Lawrence — precaudal 17.825 ± 0.038, and caudal 44.028 ± 0.055 (143 fish, range 17–19 and 42–46 vertebrae). In both cases the mean was higher for the Gulf of St. Lawrence; however, the difference between the means was significant for the precaudal ($P = 0.001$) but not for the caudal vertebrae.

The numbers of precaudal and caudal vertebrae were negatively correlated: $r = -0.339$, $P < 0.001$, for the west Greenland sample, and $r = -0.245$, $0.005 > P > 0.001$, for the Gulf of St. Lawrence sample.

The mean precaudal vertebral number for west Greenland is similar to that of Hubbs and Wilimovsky (1964) for 88 fish taken in 1925 from this region (17.64) and the caudal mean slightly but not significantly higher than that of Hubbs and Wilimovsky for the 88 fish (43.80). (This mean is incorrectly given as 44.84 (including two additional Ungava Bay fish) in Hubbs and Wilimovsky, 1964, table 6.) The greater difference between the means of samples from the Gulf of St. Lawrence and from the remainder of the area for precaudal than caudal vertebrae is unusual and is opposite to the considerably greater difference in numbers of caudal than of precaudal vertebrae shown for Pacific and Atlantic Greenland halibut (Hubbs and Wilimovsky,

1964). However, within the Pacific area itself the means of precaudal vertebrae varied more widely than those of caudal vertebrae (Hubbs and Wilimovsky, 1964).

Hubbs and Wilimovsky (1964) also found a negative correlation between numbers of caudal and precaudal vertebrae ($r = -0.49$) for a sample of 88 Greenland halibut taken in 1925 at west Greenland.

In 20 specimens from the east coast of Iceland (Schmidt, 1904) there was a range of 17–19 and a mean of 18.000 ± 0.072 for precaudal vertebrae (vertebrae without lower arches) and a range of 43–45 and a mean of 44.000 ± 0.145 for caudal vertebrae. The mean for precaudal vertebrae from east Iceland differs from that from west Greenland, 1965 ($P < 0.001$) but the means for caudal vertebrae from these two areas do not differ significantly.

Fused Vertebrae

In 2641 Greenland halibut vertebral columns, the frequency of the numbers of fused vertebral centra in one fish (number of fish in parentheses) was 2(39), 3(2), 4(6), 5(1), 7(1), and 10(1). When each partially developed fused centrum was counted as one complete vertebra the vertebral means for the 2591 fish with normal and the 50 with fused vertebrae were 61.59 ± 0.014 and 61.56 ± 0.115, respectively, and were not significantly different. The vertebral means were, therefore, not distorted by the exclusion of the small number of vertebral columns with fused vertebrae.

This finding is similar to that of Ford and Bull (1926) for Atlantic herring, *Clupea harengus*. The results of Schnakenbeck (1931), Kändler (1932), and Templeman (1948) are similar for this type of abnormality.

There was no considerable localization of fusions in the total frequency of fused vertebrae except toward the posterior end of the vertebral column (Fig. 2), especially in the last two vertebral centra (Table 3). There were no fusions of centra in vertebrae 1–15 of females, whereas in males there were 17 fusions from seven fish in this region. Based on the number of fish with fusions, reduced to 6.6 on the basis of equality of number of males and females (Table 4), a chi-square test indicates that the difference is likely to be real ($P < 0.01$), but the small numbers leave the matter open to doubt.

Similarly in the posterior part of the vertebral column from vertebral centra 59–63 (Fig. 2) there were nine fusions from five males and 18 fusions from 10 females, or considering the number of fish with fusions and equalizing for the number of males and females possessing these numbers of centra (1181 males and 1125 females) the fish numbers with fusions for equal numbers of males and females become five males and 10.5 females. A chi-square test shows that the difference is not significant.

After adjusting for equality of sexes, neither the difference in number of males and females with fusions nor that in the numbers of fused centra in the whole vertebral column (Table 4) is significant.

Itazawa (1963) showed that in the carp, *Cyprinus carpio*, and in the snakehead fish, *Channa argus*, a few vertebrae just anterior to the urostyle ossified

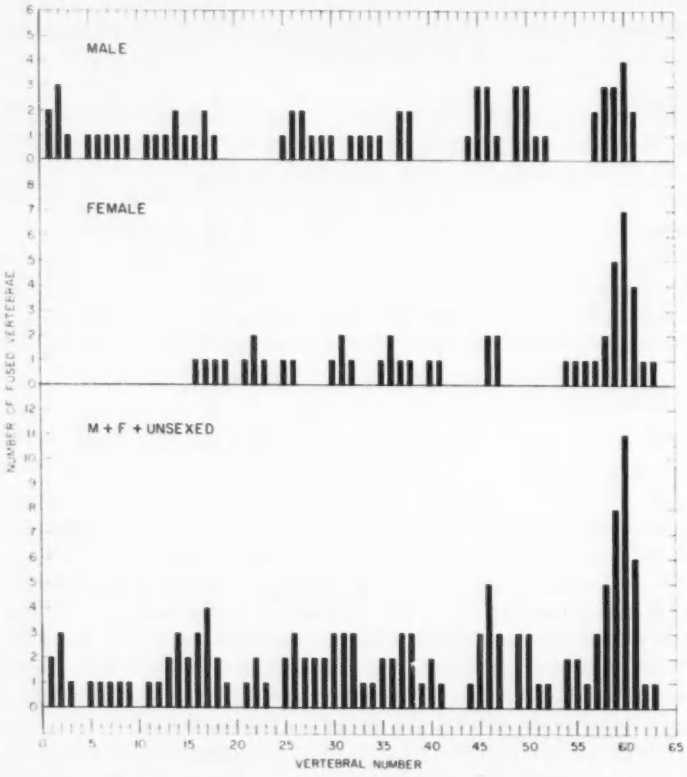

FIG. 2. Distribution of fused vertebral centra along the vertebral column of Greenland halibut from the northwest Atlantic 1950–66.

latest. There may be, however, concentrations of vertebral fusions in other regions of the vertebral column (Ford and Bull, 1926), possibly related to differential movement of fin-ray bases in relation to vertebrae during development.

The small concentration of abnormalities at vertebrae 14–18 may be related to developmental features associated with the change from precaudal to caudal vertebral types. The precaudal vertebrae usually end at vertebrae 17 and 18.

RELATION OF VERTEBRAL NUMBERS TO TEMPERATURES DURING DEVELOPMENT

The Greenland halibut has a large egg and the number of vertebrae is very likely determined before or soon after hatching (see Templeman and Pitt, 1961, for review). The similar mean numbers of vertebrae for the various areas of the northwest Atlantic are associated with similar temperatures at

TABLE 3. Fusions in the posterior part of the vertebral column of Greenland halibut from the northwest Atlantic 1950–66. The urostylar half vertebra is not included. Each centrum fused with another centrum is counted as one.

Location of fusion: no. vertebral centra from anterior end	No. fused centra in fish with 58–63 vertebral centra					Total fused centra	No. of fish, A, with number of normal + fused vertebral centra at left	No. of fish with exact number of normal + fused vertebral centra at left	% fused of A
	58	59	60	61	63				
54	–	–	2	–	–	2	2641	–	0.08
55	–	–	2	–	–	2	2641	–	0.08
56	–	1	–	–	–	1	2641	–	0.04
57	1	1	–	1	–	3	2641	–	0.11
58	1	–	2	2	–	5	2641	3	0.19
59	–	–	7	1	–	8	2638	81	0.30
60	–	–	5	6	–	11	2557	1125	0.43
61	–	–	–	6	–	6	1432	1224	0.42
62	–	–	–	–	1	1	208	202	0.48
63	–	–	–	–	1	1	6	6	16.67

TABLE 4. Fused and normal centra in vertebral columns of Greenland halibut from the northwest Atlantic 1950–66.

	Male	Female	Unsexed	Total
No. vertebral columns	1183	1126	332	2641
No. with fused vertebral centra	26	21	3	50
No. fused centra	67	49	14	130

the depths of 600–1200 m at which the eggs and yolk-sac larvae probably develop.

The Greenland halibut does not spawn in the colder waters of the west Greenland fjords where it is plentiful, nor to the north of the Davis Strait Ridge, although it is numerous there, but migrates outward and southward to spawn south of the Davis Strait Ridge in the deep parts of Davis Strait south of 66°45′N (Jensen, 1935). Captures of early larvae 10–16 mm long (many with yolk sac, showing that they had recently hatched) in May off west Greenland at about 600–1000 m in water about 680–1660 m deep indicate that the spawning is in deep water, the eggs floating and hatching in the deeper layers. As the larvae become larger they rise toward the surface.

Smidt (1969) reports for west Greenland that a few large pelagic eggs, probably of Greenland halibut, were taken in March-May in the upper layers, some in the upper 50 m, but that 1500 large pelagic eggs of the same size, and possibly Greenland halibut, were taken in one 2-m stramin-net haul in April with 1500 m wire out, west of Fylla Bank although no eggs were taken in a surface haul at the same station.

The deepwater west Greenland spawning areas south of the Davis Strait Ridge are in water with a temperature of 3.5–4.5 C, whereas the corresponding

depths north of the ridge, where spawning does not occur, have almost everywhere a temperature less than 2 C (Jensen, 1935).

In many sections on the west Greenland slope south of the Davis Strait Ridge in March–October 1928–35, temperatures declined from about 5 to 4 C at 600 m to between 4 and 3 C at 900–1500 m. On the continental slope from the southern end of Baffin Island to the northern Grand Bank, temperatures in July–September 1928, 1931, and 1933 were about 4.0–3.2 C at 600 m, 3.6–3.1 C at 1200 m, and 3.2–3.0 C at 1500 m. Temperatures east of the Grand Bank at these levels were close to these ranges (Smith et al., 1937).

These areas and depths with moderately warm deep water from west Greenland and Baffin Island to eastern Newfoundland and the northern Grand Bank cover the main areas of concentrations of Greenland halibut and in all probability the main spawning areas and depths.

The ovaries of Greenland halibut from the northern Gulf of St. Lawrence were maturing in November. These fish could not be expected to leave the gulf through the shallow Strait of Belle Isle. Consequently, unless they migrate out of the gulf through the Laurentian Channel, they presumably spawn in the channels of the gulf where the depths are much shallower than on the spawning grounds of the outer slopes. Although the bottom water temperatures in these channels are suitably high, the layer of warm water between the depths with temperatures suitable for spawning and the overhead layer of water below zero centigrade is a shallow one, and the developing eggs may come under the influence of the colder water of the overhead layer, which might increase the number of vertebrae.

Schmidt (1904) in 20 Greenland halibut taken east of Iceland in 1904 found a vertebral range of 61–63 and mean of 62.008 ± 0.145. There is no indication whether he was including the urostylar half vertebra as a vertebra but from the high count he presumably was. This mean for east Iceland is different ($P = 0.005$) from my means for west Greenland (Table 1, 61.584 ± 0.034) and for the whole Canadian area apart from the Gulf of St. Lawrence (61.574 ± 0.015) but the smallness of the sample from east of Iceland indicates the necessity for caution in dealing with these data. Temperatures at depths frequented by Greenland halibut are considerably lower east of Iceland (Stefánsson, 1962) than off the southern part of the west Greenland coast and in the Canadian area. However, judging from the higher temperatures in the west Greenland spawning areas (Jensen, 1935) and the temperature distribution in deep water around Iceland (Stefánsson, 1962) the Greenland halibut found on the east coast of Iceland were probably derived from spawning in warmer waters such as occur off the west coast of Iceland.

NUMBERS OF FIN RAYS

In Dorsal and Anal Fins

Although the dorsal fin-ray means for the Newfoundland area tended to be lower than that for west Greenland, there were no significant differences

between the means for the various areas, which range from 96.889 to 98.026 (Table 5).

The differences between the anal fin-ray means (72.888–73.474) of samples 5–10 (Table 5) from west Greenland to the southern Grand Bank are not significant at the 5% level. The mean (71.864) for the small sample 11 from the Flemish Cap deep water differed from those (73.207–73.474) for samples 7–9 ($P = 0.05$ to <0.025) and from that for the Newfoundland area (73.318, total of samples 6–10, $P = 0.02$), but did not differ significantly from those (72.888–73.056) for samples 5 and 6.

In the Flemish Cap area, Greenland halibut are scarce, temperatures are generally higher at the intermediate levels than in other Newfoundland areas where Greenland halibut are found, and the intermediate layer of very cold water generally found in the Newfoundland area is absent. This might tend to give lower fin-ray averages to developing larvae at Flemish Cap if they were at these levels. The smallness of the Flemish Cap sample and the lack of significant differences between the dorsal fin-ray mean of this sample (Table 5) and those from other areas of the northwest Atlantic render doubtful the apparent significance of the differences between the low Flemish Cap anal fin-ray mean and those of neighbouring areas.

Boyar (1964) for four specimens of Greenland halibut taken from the southern end of its range, in the Gulf of Maine in 1963, reported 96–98 dorsal and 72–74 anal rays, and Norman (1934) reported ranges of dorsal fin rays 94–102 (mean 92) and of anal fin rays 71–76 for 14 Greenland halibut, 12 from northern Iceland, 1 from Greenland, and 1 from southwest of Ireland. These are within the ranges of 90–107 and (62) 66–84 for our specimens.

For 20 specimens of Greenland halibut caught east of Iceland in 1904 (Schmidt, 1904) there was a range of 95–103, mean 98.450 ± 0.639, for dorsal rays, and a range of 68–79, mean 73.000 ± 0.620, for anal rays. These averages are not significantly different from dorsal and anal-ray means from west Greenland (Table 5) nor from those for the total of the Canadian area.

The dorsal and anal fin-ray means for west Greenland for 102 specimens from ca. 1898 to 1928 (Hubbs and Wilimovsky, 1964; allowing for the addition of one fin ray because of the method of counting by these authors) are 97.529 ± 0.386 and 73.167 ± 0.285. Neither of these is significantly different from the west Greenland dorsal and anal fin-ray means for 1965 (Table 5).

In Pectoral Fins

In a sample of 64 Greenland halibut from west Greenland in 1965 (70° 02′–63°28′N, 53°00′–60°57′W, 650–1460 m) the distribution of fin-ray number in the right pectoral (number of fish in parentheses) was 12(4), 13(24), 14(34), 15(2), with a mean of 13.531 ± 0.083, and in the left pectoral 13(8), 14(7), with a mean of 13.467 ± 0.133, no significant difference between the averages.

These results are not significantly different from the 13.485 ± 0.060 for the right pectoral (103 fish) and 13.573 ± 0.072 for the left pectoral (82

TABLE 5. Numbers of dorsal and anal fin rays in Greenland halibut from the northwest Atlantic.

	Area	Year	Depth (*m*)	Bottom temp (*C*)	Total no. fish	No. fin rays	
						Mean ± SE[a]	Range
	In dorsal fins						
1.	W Greenland 70°10′15″–63°28′N 53°00′–60°57′W	1965	630–1460	0.21–4.57	78	98.026±0.456	90–106
2.	S slope Grand Bank 44°12′30″–42°50′30″N 50°40′–52°56′W	1966	64–295	2.24–3.58	69	97.667±0.409	90–106
3.	Flemish Cap 48°58′–45°48′N 43°45′–46°26′W	1968	1370–1630	3.57–3.66	22	97.000±0.798	91–107
4.	S slope Grand Bank 44°15′–43°44′30″N 48°51′–53°17′W	1968	1460–1600	3.75–3.98	9	96.889±1.476	90–104
2–4.	Flemish Cap–S Grand Bank 48°58′–42°50′30″N 43°45′–53°17′W	1966–68	64–1600	2.24–3.98	100	97.450±0.355	90–107
	In anal fins						
5.	W Greenland 70°10′15″–63°28′N 53°00′–60°57′W	1965	630–1460	0.21–4.57	80	72.888±0.337	62–80
6.	Off Belle Isle, NE Nfld. shelf 51°55′N 53°48′W	1950	362–375	2.47	36	73.056±0.496	67–80
7.	Badger Bay 49°25′30″N 55°39′W	1951	285–295	0.13	114	73.474±0.278	68–84
8.	Trinity Bay 48°08′N 53°13′W	1953	307–351	0.33–0.39	111	73.207±0.258	67–79
9.	S slope Grand Bank 44°12′30″–42°50′30″N 50°40′–52°56′W	1966	64–295	2.24–3.58	78	73.372±0.321	66–80
10.	E and SW slopes Grand Bank 44°15′–43°44′30″N 48°51′–53°17′W	1968	1460–1600	3.75–3.98	10	73.300±0.775	69–76
6–10.	NE Nfld. shelf to S Grand Bank 51°55′–42°50′30″N 48°51′–55°39′W	1950–68	64–1600	0.13–3.98	349	73.318±0.152	66–84
11.	Flemish Cap 48°58′–45°48′N 43°45′–46°26′W	1968	1370–1630	3.57–3.66	22	71.864±0.600	68–79

[a]SE = standard error.

fish) of Hubbs and Wilimovsky (1964) for the same area, but the difference (nonsignificant) between the two pectorals is reversed. This is an indication of the similarity between the pectoral fin-ray numbers of both sides.

RELATION BETWEEN NUMBERS OF ANAL AND DORSAL FIN RAYS AND THOSE OF VERTEBRAE

For occasional random samples from the area from west Greenland to the southern Grand Bank, there was a high correlation between numbers of anal and dorsal fin rays in 173 specimens ($r = 0.706$, $P < 0.001$) but no significant correlation between numbers of dorsal fin rays and vertebrae in 150 specimens ($r = 0.108$), nor between numbers of anal fin rays and vertebrae in 387 specimens ($r = 0.066$).

My results are similar to those of Hubbs and Wilimovsky (1964) for a sample of 83 specimens 76–103 mm long from west Greenland.

Vertebral number in fishes may be determined earlier in development than the number of dorsal and anal fin rays, and the numerical effects of a particular temperature may be different for these fin rays and vertebrae (Tåning, 1952). The numbers of vertebrae, therefore, are not necessarily closely related to those of dorsal and anal fin rays.

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Lactate Dehydrogenase Systems of Rainbow Trout — Evidence for Polymorphism in Liver and Additional Subunits in Gills

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WILLISCROFT, S. N., AND H. TSUYUKI. 1970. Lactate dehydrogenase systems of rainbow trout — evidence for polymorphism in liver and additional subunits in gills. J. Fish. Res. Bd. Canada 27: 1563–1567.

Three lactate dehydrogenase phenotypes, CC, CC′, and C′C′, were encountered in the livers of rainbow trout (*Salmo gairdneri*) resident in Kokanee Creek, which flows into the west arm of Kootenay Lake, B.C. The rare subunit C occurred in high frequency in specimens from this area. Two additional lactate dehydrogenase subunits, designated E^1 and E^2 in this study, and not reported previously, were found in the gill tissues of common stocks of rainbow trout homozygous for the C′C′ phenotype, raising the total number of molecular species, based on 10 electrophoretically distinct subunits, to at least 23 for this species.

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INTRODUCTION

LACTATE DEHYDROGENASE (LDH) in fishes has been used as genetic markers for the investigation of several biological problems. This is partly because its tetrameric structure (Markert and Møller, 1959), its ability to dissociate and hybridize (Appella and Markert, 1961; Markert, 1963, 1968) without appreciable loss in enzymic activity, and its occurrence in nature in many variant forms have been well documented. In salmonids, e.g., LDH (Klose et al., 1967; Ohno et al., 1968; Massaro and Markert, 1968) and, later, malate dehydrogenase (Bailey et al., 1969) and blood hemoglobins (Tsuyuki and Ronald, 1970) provided convincing evidence that many salmonid species are tetraploid by gene duplication. Interspecies comparisons (Markert and Faulhaber, 1965; Nakano and Whiteley, 1965; Daugherty, MS, 1966; Bailey and Wilson, 1968; Lush et al., 1969) and ontogenetic variations (Nakano and Whiteley, 1965; Daugherty, MS, 1966; Hitzeroth et al., 1968; Goldberg et al., 1969) of LDH have also been studied. Genetic variants have been reported in the A subunits of Pacific hake (*Merluccius productus*) (Utter and Hodgins, 1969), northern anchovy (*Engraulis mordax*) (Ohno et al., 1968), the B subunits of silver hake (*Merluccis bilinearis*) (Markert and Faulhaber, 1965), brook trout (*Salvelinus fontinalis*) (Morrison and Wright, 1966), herring (*Clupea harengus harengus*) (Odense et al., 1966), cod (*Gadus morhua*) (Odense et al., 1969; Lush, 1970), coalfish (*Gadus virens*) (Lush, 1970), sockeye salmon (*Oncorhynchus nerka*) (Hodgins et al., 1969), longnose dace (*Rhinichthys cataractae*)

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(Clayton and Gee, 1969), and in both the A and B subunits of hagfish (*Eptatretus stoutii*) (Ohno et al., 1967). Some of these LDH variants, e.g., herring (Odense et al., 1966), cod (Odense et al., 1969), sockeye salmon (Hodgins et al., 1969), and Pacific hake (Utter and Hodgins, 1969) have been used effectively in population analysis investigations.

In the salmonids, the brook trout possessed three phenotypes (Goldberg, 1966; Morrison and Wright, 1966) consisting of two homozygous, five-banded systems of different mobilities, and a nine-banded heterozygous form, which originated from the presence of an allelic variant of the B subunit. A genetic analysis of the LDH systems of the splake, a fertile hydrid between brook trout and lake trout (*S. namaycush*) (Stenton, 1950), is also reported in these studies, and certain tissue isozymes of the brook trout have been described by Bouck and Ball (1968) and by Hochachka (1966), who indicated the presence of five subunits resulting in 14 molecular species of the enzyme. Lactate dehydrogenase systems of the rainbow trout (*Salmo gairdneri*) have been reported to possess 15 (Bouck and Ball, 1968) and 18 (Massaro and Markert, 1968) isozymes. Some inconsistency, therefore, appears to exist both in the numbers and the relative mobilities of the isozymes of this species. The present report, in describing a variant subunit in the liver and two in the gill tissues, not only demonstrates the first occurrence of LDH polymorphism in the liver of rainbow trout, but also increases the number of molecular species.

MATERIALS AND METHODS

Rainbow trout were obtained from Kokanee Creek near Nelson, B.C., and from the Sun Valley Trout farm in Abbotsford, B.C. Tissues were used either frozen or fresh.

Most tissue extracts were prepared by homogenizing tissue with four volumes of chilled sodium phosphate buffer of *p*H 7.55 and ionic strength of 0.05. Intestinal tissue was homogenized with three volumes of buffer and eye tissue was used without further addition of water. The clear supernatant from centrifuging was applied directly to the starch gel.

The buffers used by Clayton and Gee (1969), consisting of 0.002 M citric acid adjusted to *p*H 8.0 with tris (hydroxymethyl) amino methane (Tris), offered by far the clearest resolution for the LDH of rainbow trout. The starch gel was prepared as a 13% solution in this buffer. The electrode solution was made of 0.04 M citric acid brought to the same *p*H with Tris. Electrophoresis was carried out at 4 C at 200 v for 2½ hr as described previously (Tsuyuki et al., 1968).

The lactate dehydrogenase was made visible histochemically at room temperature by submersion for 30 min in a minimal volume of a reaction mixture consisting of the following (Morrison and Wright, 1966) in 100 ml final volume: 20 ml of 0.1 M Tris adjusted to *p*H 8.0 with HCl, 0.96 g of lithium lactate, 30 mg of nicotinamide adenine dinucleotide, 1.5 mg of phenazine methosulfate, and 20 mg of nitrobluetetrazolium. The gels were washed with water, photographed, and reexamined after 24 hr for development of any minor components.

RESULTS AND DISCUSSION

LDH isozymes were observed in seven tissues of the rainbow trout (Fig. 1, 2). Evidence suggests 10 different subunits (Fig. 2) accounting for up to 23 molecular species of LDH found in rainbow trout. If the faint suggestion of what appears to be the "*d*" series described by Massaro and Markert (1968) that we found in the liver, brain, and eye tissues only after massive quantities of samples were used, are included, this number will be increased. Using the

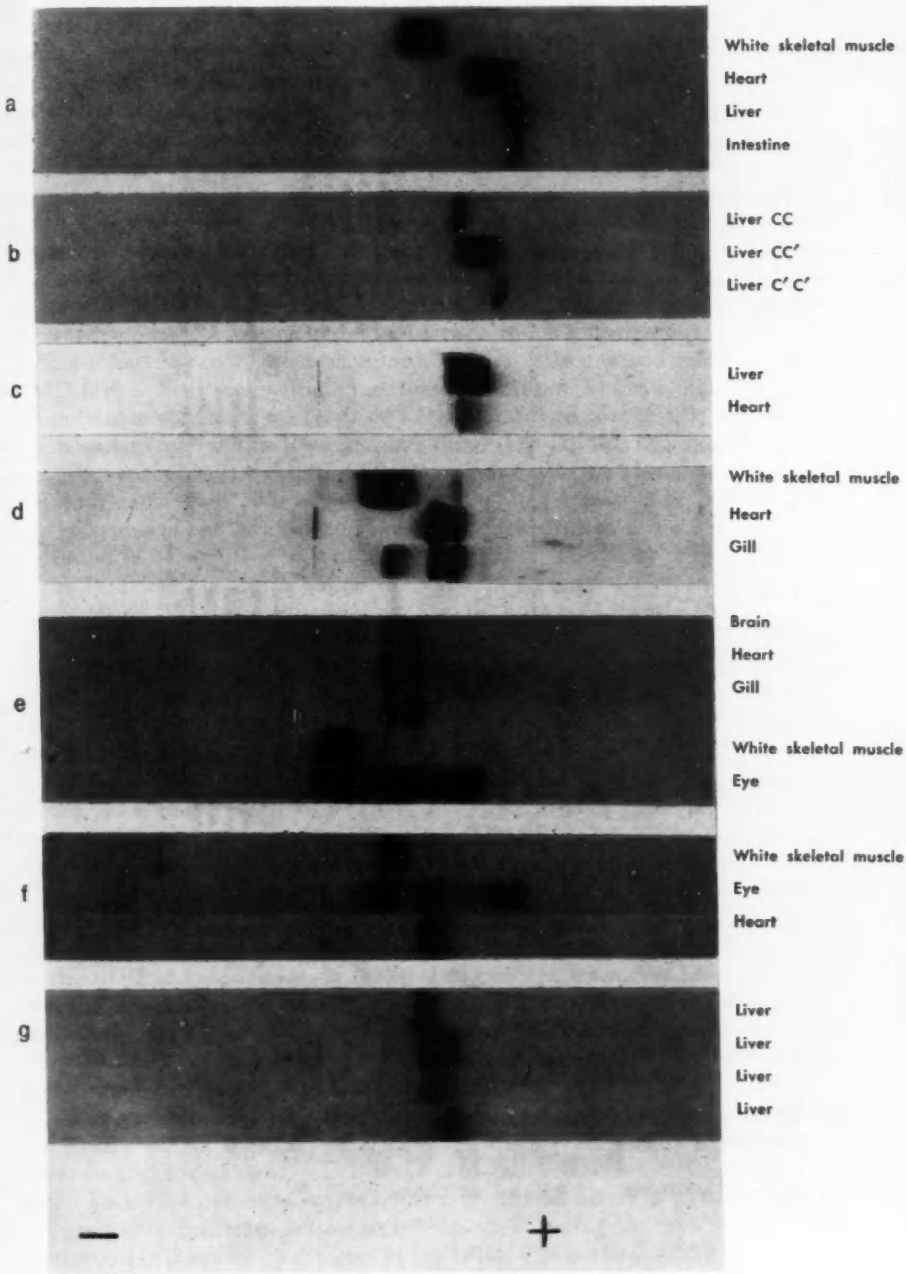

FIG. 1. Starch gel electrophoretic zymograms of LDH from the various tissues of rainbow trout.

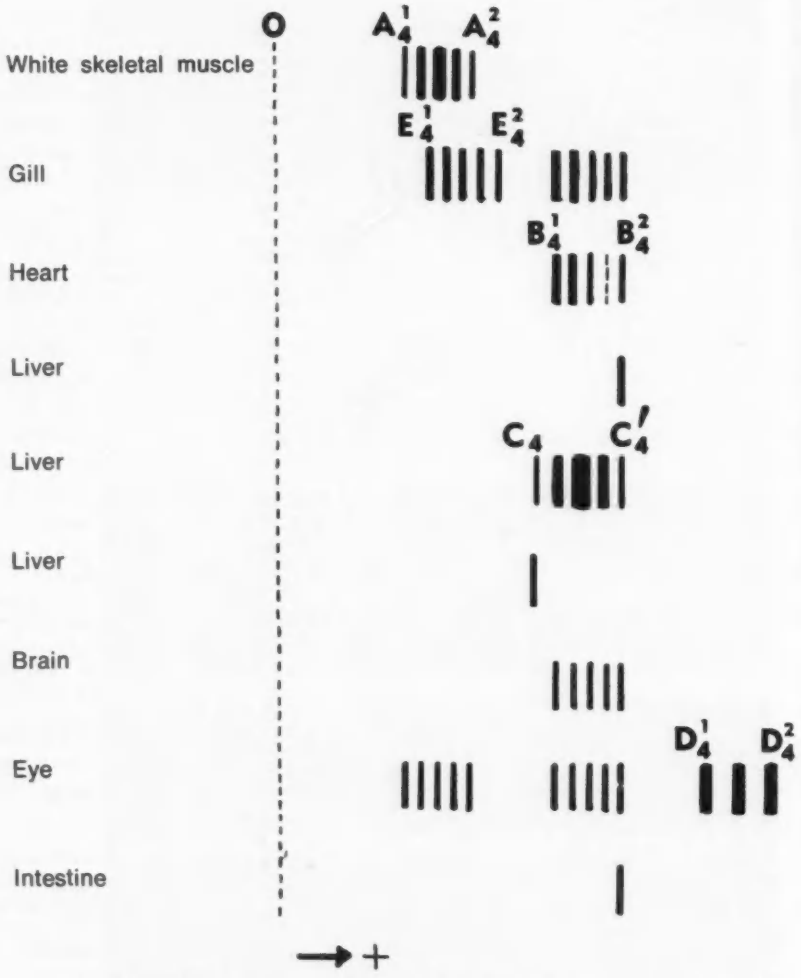

FIG. 2. Schematic representation of molecular species of rainbow trout LDH resulting from subunits from white skeletal muscle (A^1, A^2), heart muscle (B^1, B^2), liver (C, C′), eye tissue (D^1, D^2), and gill tissue (E^1, E^2).

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basic nomenclature adopted by Markert (1962) and extended by Odense et al. (1969), these polypeptides were designated (Fig. 2) A^1 and A^2 in white skeletal muscle, B^1 and B^2 in heart muscle, D^1 and D^2 in the eye tissue, and E^1 and E^2 in the minor components of gill tissue. Although the subunit pairs in the above tissues are indicative of products from separate gene loci, the pair from liver are allelic variants of a single locus and are, therefore, designated C and C′. The liver tissue was unique in that the LDH existed in three phenotypic forms (Fig. 1*b*), consisting of two single-zoned homotetramers, C_4 and C'_4, from homozygous individuals and a five-zoned system in approximately binomial proportions consisting of the two homotetramers and three heterotetramers of intermediate mobilities, from heterozygous individuals. In more than 100 individuals examined, the liver tissue was practically devoid of all LDH activity zones other than three phenotypes. The eye tissue displayed 13 molecular species (Fig. 1*e*, *f*) of LDH, comprised of the five isozymes of the A series, five of the B series, and three (D^1_4, D^1_2, D^2_2, D^2_4) specific for eye in agreement with the results of Morrison and Wright (1966) and Massaro and Markert (1968). The white muscle (Fig. 1*a*) consistently possessed the five izozymes of the A series and invariably lacked those of the B series, which are found in heart tissue as well as in red muscle. The heart muscle consisted of the B series (Fig. 1*a*) in proportions similar to those described by Massaro and Markert (1968). Intestinal tissue (Fig. 1*a*) possessed a single zone, identical in mobility to the C'_4 homotetramer of liver. The brain tissue (Fig. 1*e*) displayed isozymes with mobilities seemingly identical to the B series, but in different proportions, and also traces of those peculiar to the gill tissue. The major isozymes of the gill tissue (Fig. 1*d*, *e*) appear similar to the B series; the minor components represent a new E series.

Polymorphism in the liver LDH of rainbow trout has not been described before. The single-zoned liver LDH of *Salmo irideus* (described by Klose et al., 1968), and in rainbow trout, the LDH designated D_4 by Massaro and Markert (1968), isozyme E of group 2 described by Bouck and Ball (1968), and the liver isozyme described by Bailey and Wilson (1968) all appear to correspond to isozyme C'_4 in Fig. 2. Hochachka (1966) also indicated the presence of only a single-zoned homotetramer, of uncertain mobility in relation to the LDH of heart tissue, in the liver of rainbow trout. We have also found that the common hatchery stock of rainbow trout employed in this area possess exclusively the C'_4 homotetramer in the liver tissue. Other than in occasional trace amounts, in no instance was an isozyme encountered in the liver tissue with anodal mobility greater than that of C'_4.

The variant rainbow trout with the C_4 homotetramer is thus rare, but was found widely distributed, along with those possessing the common C'_4 type, in Kokanee Creek, which flows into the west arm of Kootenay Lake, B.C. This rare homozygote apparently originated above a natural waterfall impassable to upstream migrants, for the allele coding for the C subunit predominated in this area (Northcote et al., 1970). The possibility that yellowstone cutthroat trout (*Salmo clarki lewisi*) entered the gene pool was discounted

as this species possesses exclusively the isozyme with a mobility identical to the C_4' homotetramer. Phenotypic distribution of the two homozygotes and the heterozygote agreed well with the Hardy-Weinberg proportions.

Although the electrophoretic mobility of homotetramer B_4^2 and C_4' are virtually identical, subunits B^2 and C' are considered to be different. Artificial hybridization of liver C_4 and C_4' produced a five-zoned pattern in approximately binomial proportions identical to that of the heterozygote (Fig. 1g) although preliminary results indicated that C_4, and by implication C_4', could not be hybridized with heart muscle LDH. This lack of intergroup hybridizability was pointed out by Massaro and Markert (1968) who likewise stated that isozymes designated B_4' and D_4 in their study have identical mobilities, but are different by virtue of the inability of the D subunit to hybridize with LDH-5 from mammalian sources.

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Elimination of Bacteria by the Northern Quahaug (*Mercenaria mercenaria*): Environmental Parameters Significant to the Process[1]

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Heffernan, W. P., and V. J. Cabelli. 1970. Elimination of bacteria by the northern quahaug (*Mercenaria mercenaria*): environmental parameters significant to the process. J. Fish. Res. Bd. Canada 27: 1569–1577.

A study was conducted to determine the influence of various environmental parameters on the ability of the northern quahaug, *Mercenaria mercenaria*, to cleanse itself of artificially accumulated *Escherichia coli*. The elimination of *E. coli* to low residual levels in the quahaugs placed in sea water free of *E. coli* for 48–72 hr was dependent on their initial *E. coli* content and, hence, the level of the organism in the contaminating water. Extending the contamination interval from 6 hr to 14 days did not adversely affect the ability of the animals to cleanse themselves of the organisms they accumulated. Optimal elimination was obtained within the following environmental limits: temperature, 10–20 C; salinity, 22–31‰; flow rate, > 13 ml/min per animal; turbidity, trace to 25 Jackson Turbidity Units. When the ambient temperature of the water from which the animals were collected was less than 10 C, the elimination of *E. coli* was decreased. Preliminary data were presented to demonstrate the importance of examining the variability in the response of individual animals.

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It is well established that bivalve molluscs taken from polluted waters, through their ability to concentrate microorganisms from their environmental sea water (Kelly, 1956; Metcalf and Stiles, 1965; Liu et al., 1966; Cabelli and Heffernan, 1970a), can and have served as vehicles for the transmission of enteric diseases to man by their consumption in the raw state (Hart, 1945; Dougherty and Altman, 1962). Conversely, artificially or naturally polluted shellfish will markedly reduce their content of enteric microorganisms when functioning in a seawater environment free of these microorganisms. Studies documenting this process and the environmental parameters relevant to it have been performed with the oyster (Wells, 1916; Wood, 1961; Hedstrom and Lycke, 1964; Mitchell et al., 1966), the soft shell clam (Arcisz and Kelly, 1955; Erdman and Tennant, 1956), and the mussel (Dodgson, 1928; Crovario, 1958). The commercial application of this process to the purification of shellfish taken from polluted waters (depuration) has been existent in Great Britain and continental Europe for some time.

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In most respects, depuration is a specialized application of the normal feeding mechanism of the animal and, therefore, is mediated by such physiological functions as pumping rate, filtration efficiency, the rate of transport and elimination of materials through the digestive system, etc. These functions, in turn, can be influenced by a variety of environmental parameters such as the temperature and salinity of the water, the nature and quantity of suspended particulates and dissolved solids and gases therein, the level and duration of contamination in the animals, and finally, the environmental history of the animals.

In the study to be described on the elimination of *Escherichia coli* from artificially contaminated quahaugs, *Mercenaria mercenaria*, several of the above parameters were considered. *Escherichia coli* was chosen as the model organism to describe the elimination of bacteria by the quahaug because it is a part of the group of organisms whose removal could be used in monitoring the depuration process.

MATERIALS AND METHODS

The techniques for the preparation of the *E. coli* cultures for contamination of the animals, the sources of sea water and quahaugs, and the arrangement of the experimental flow-through system were those described previously (Cabelli and Heffernan, 1970a). Seawater and shellfish samples were assayed using a modification of the MacConkey agar roll tube method of Clegg and Sherwood (1947). The formula for MacConkey agar as available from commercial sources was modified by decreasing the bile salts concentration from 0.15 to 0.075%. The sodium chloride eliminated from the medium essentially was replaced by that present in the shellfish and seawater samples mixed with it. The formula for the single-strength medium, as used, was as follows (grams per liter): peptone, 17.0; polypeptone, 3.0; lactose, 10.0; bile salts #3, 0.75; agar, 13.5; neutral red, 0.03; crystal violet, 0.001. The ingredients of the medium, in an appropriate quantity of distilled water, were boiled for 10 min, and the medium was held in a water bath at 55–60 C; it was used within 6 hr of preparation.

Quahaug samples were prepared for assay by homogenizing the required number of animals in a Waring Blendor for 60 sec at 18,000 rpm and adding 6 g of the homogenate to a 6-oz bottle containing 54 ml of sterile, phosphate-buffered saline (buffered water to which 0.9% NaCl was added). A 60-ml amount of double-strength medium was added to the bottle, the contents of which were mixed gently and distributed into six petri dishes (100 × 15 mm). When required, water samples were assayed by adding 60 ml of double-strength medium to 60 ml of the seawater sample in a sterile 6-oz bottle. The contents were mixed gently and distributed to six petri dishes as above. The medium was allowed to solidify, and the inverted plates were incubated in air at 45.5 ± 0.5 C for 24 hr. At this temperature, growth of organisms other than *E. coli* present in the quahaug or the sea water was inhibited or reduced, and the *E. coli* grew as characteristic brick red, subsurface colonies. Occasionally, other coliform organisms would grow at the elevated temperature; therefore, only characteristic colonies greater than 0.5 mm in diameter were counted.

Periodically, samples of the intake water that had passed through the ultraviolet light germicidal treatment chamber were assayed to determine if they contained any viable *E. coli* cells; none were detected.

After an accumulation period of 24 hr, samples of water were taken from the tanks, and 6–24 animals were removed for assay as a pool or as individuals. The addition of *E. coli* to the water in the mixing boxes was discontinued; the tanks were drained; the animals were sprayed with sea water to reduce fecal and pseudofecal deposits adhering to the shells; and the tanks were allowed to refill with sea water. This washing and assay procedure was repeated every 24 hr during the course of each experiment. The animals removed for assay were replaced by marked, uncontaminated quahaugs.

The temperature of the environmental water was varied by mixing appropriate concentrations of ambient and heated or cooled water. Mixtures of fresh water and sea water were used to vary the salinity experimentally.

The following environmental conditions were maintained during uptake and, with the exception of the particular parameter under study in a given experiment, during elimination as well: flow rate, 10 ml/min per animal; temperature, 20 C; salinity, 30–31‰. Unless noted to the contrary, the experiments were performed between late May to early October when the ambient temperatures of the waters from which the animals were taken were between 15 and 22 C.

RESULTS

Although the accumulation studies reported previously (Cabelli and Heffernan, 1970b) showed that maximum accumulation of *E. coli* in active animals occurred within 6 hr of contamination, the possibility did exist that, subsequently, some of the organisms were translocated to sites less amenable to elimination. Therefore, experiments were performed to document depuration as a consequence of accumulation periods up to 14 days. To accomplish this, groups of animals were placed in one of the tanks such that at the end of 14 days four groups of animals had been permitted to accumulate the organisms for 1, 3, 7, or 14 days, respectively. The suspension of organisms fed into the mixing tank was changed every 48 hr. The levels of *E. coli* in the water varied between 10^2 and 10^4/100 ml. The quahaugs then were placed in separate tanks, and the elimination phase of the experiment was started. After a 48-hr treatment interval, numbers of *E. coli* in excess of the sensitivity of the assay method (17 colony-forming units (CFU)/100 g) were not recovered from any of the four groups. Thus, in subsequent experiments, no need was seen to extend the accumulation period beyond 48 hr because the accumulation interval did not appear to influence subsequent depuration.

The influence of the temperature of the water used in the flow-through system on the elimination process was examined in several experiments in which temperatures of 10 and 20 C were maintained in the tanks. From Fig. 1 it can be seen that there was no appreciable effect of temperatures between 10 and 20 C on the elimination of *E. coli* in the quahaug.

Quahaugs placed in waters having salinities of 31 and 25‰ rapidly reduced their *E. coli* content within 48 hr. However, at 20‰, poor elimination was obtained. These data, also shown in Fig. 1, confirmed visual observations on shell closure as the salinity was gradually decreased over an interval of 1 week. When the salinity reached 22‰, most of the quahaugs were observed to close. After a 4-week adaptation period in 20‰ water, most of the animals were found to be active at this salinity. After these animals were allowed to accumulate *E. coli* from an environmental water level of 4.9×10^3 *E. coli* per 100 ml, elimination proceeded equally well at salinities of 20, 25, and 30‰; however, at 15‰, elimination again was protracted (Table 1).

The rate at which the water was fed into depuration tanks was examined in several experiments. It can be seen from Fig. 1 that at flow rates below 13 ml/min per animal (approximately 1 gal/min per bu), elimination of the organisms was significantly reduced.

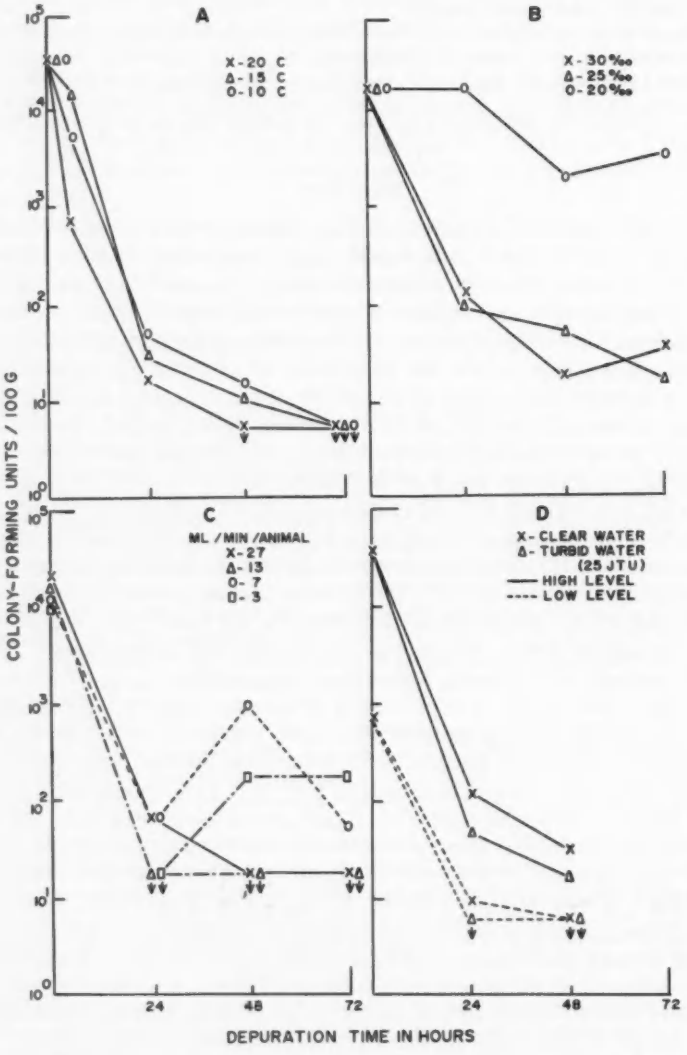

FIG. 1. Influence of (A) temperature, (B) salinity, (C) flow rate, and (D) turbidity of the environmental water on the elimination of *Escherichia coli* in artificially contaminated quahaugs. The sensitivity of the assay system was increased beyond 17 colony-forming units (CFU) per 100 g by assaying 16–18 g of shellfish homogenate per sample. The sensitivity of the assay method for the water was 1.7 CFU/100 ml. Arrows (↓) indicate levels below the sensitivity of the assay method.

TABLE 1. Effect of salinity on depuration of acclimated[a] animals at indicated salinities.

Depuration time (*hr*)	*E. coli* residual levels (CFU/100 g) × 10^2			
	30‰	25‰	20‰	15‰
0	49	49	49	49
24	0.3	<0.2	0.5	10.6
48	0.5	<0.2	<0.2	8.8

[a]Animals acclimated to 20‰ sea water prior to depuration.

It was observed that at a water turbidity of 10 Jackson Turbidity Units (JTU), achieved by the addition of bentonite to the environmental water, there was a 24% reduction in the number of animals that accumulated *E. coli* optimally. At 25 JTU only 5% of the animals accumulated the organism to levels in excess of that in the water. Elimination, however, was not decreased when the water was adjusted to a turbidity of 25 JTU; in fact, the increased turbidity appeared to have enhanced the depuration process (Fig. 1).

The time required to achieve elimination to the sensitivity of the assay method, 17 CFU/100 g, but not the initial rate of elimination, was found to be dependent on the contamination level of the animals used (Fig. 2).

Several depuration experiments with quahaugs artificially contaminated with *E. coli* were performed during late winter and early spring. At this time the ambient temperatures of the waters from which the animals were removed were less than 10 C. Only those quahaugs contaminated to levels of less than 3×10^3 CFU/100 g eliminated *E. coli* to the sensitivity of the assay method within 48–72 hr. The data, graphically shown in Fig. 3, are illustrative of the type of results obtained. Several features characterized the information obtained during the period with those animals artificially contaminated to levels in excess of 3×10^3 CFU/100 g. There was a 1–2 log reduction during the first 6 hr of depuration. However, the rate of elimination thereafter was considerably reduced and erratic. These erratic results may have arisen from considerable variability in the response of the individual quahaugs. Elimination from initial levels in excess of 3×10^3 CFU/100 g also was protracted with animals taken during and after the late fall when the water temperature had fallen below 18 C. From May 1 through May 23, 1966, four experiments with artificially contaminated animals were performed at weekly intervals. During this period, the ambient water temperatures gradually increased from about 8 to 13 C. With each successive experiment, elimination during the first 6 hr decreased, whereas that during the subsequent 18 hr increased. These observations are shown in Fig. 4.

The variability of the residual levels in individual quahaugs was examined in a series of experiments in which artificially contaminated animals were subjected to the depuration process. The results of three such trials in which

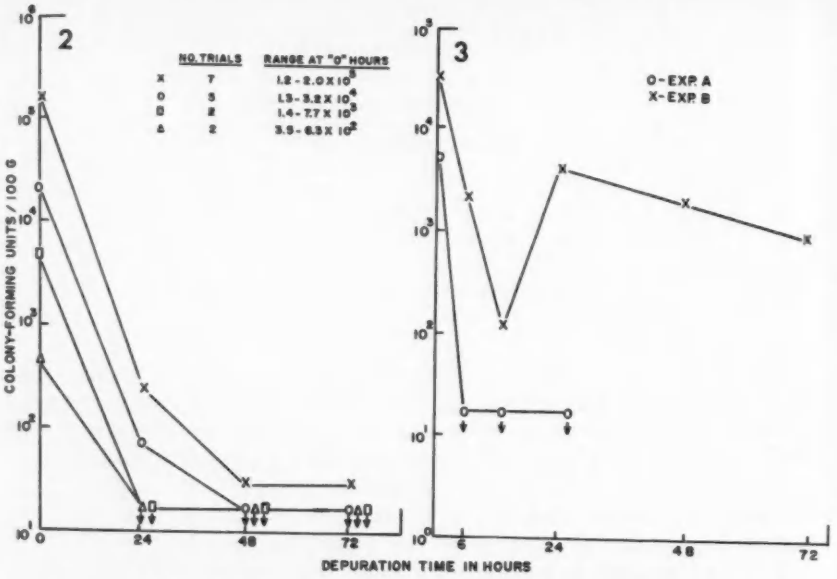
2
NO. TRIALS
RANGE AT "0" HOURS
X 7 1.2 - 2.0 X 10^5
O 3 1.3 - 3.2 X 10^4
□ 2 1.4 - 7.7 X 10^3
Δ 2 3.9 - 6.3 X 10^2
COLONY-FORMING UNITS / 100 G
0 24 48 72
3
O - EXP A
X - EXP B
6 24 48 72
DEPURATION TIME IN HOURS

FIG. 2. Elimination of *Escherichia coli* artificially introduced into the quahaug as a function of the initial contamination level in the animals. Experiments were grouped according to the initial contamination levels ("0" hr). The points for a given group represent the geometric means of the recoveries obtained at that time interval for the trials in the group.

FIG. 3. Results from typical early spring depuration trial with animals artificially contaminated with *E. coli* (see Fig. 2). Environmental conditions: temperature 20 C; salinity 30‰; flow rate 10 ml/min per animal.

the animals to be treated were contaminated to different *E. coli* levels are shown as log probability plots in Fig. 5.

DISCUSSION

The data on the elimination of *E. coli* by the quahaugs agree rather well with those produced by Liu and his co-workers (1966) at this laboratory using an identical experimental system to study Poliovirus 1 elimination in the northern quahaug. However, viral elimination appears to be somewhat more sensitive than bacterial depuration to the effect of temperatures less than 15 C.

It is of interest that the uptake of *E. coli* (Cabelli and Heffernan, 1970a, b), is much more sensitive to the effect of increased turbidity from silt, algae, etc., than is the elimination of this organism as shown herein. This is as expected since an increase in the particulate matter taken into the gut of the animal would tend to "dilute" the bacteria ingested simultaneously, thereby reducing the concentration of *E. coli* in the gut. During the elimination phase, the increased particulates passed into the gut could, if anything, speed up the elimination of *E. coli* already contained therein. There is a suggestion that the same situation prevails as regards the influence of low temperatures. Thus,

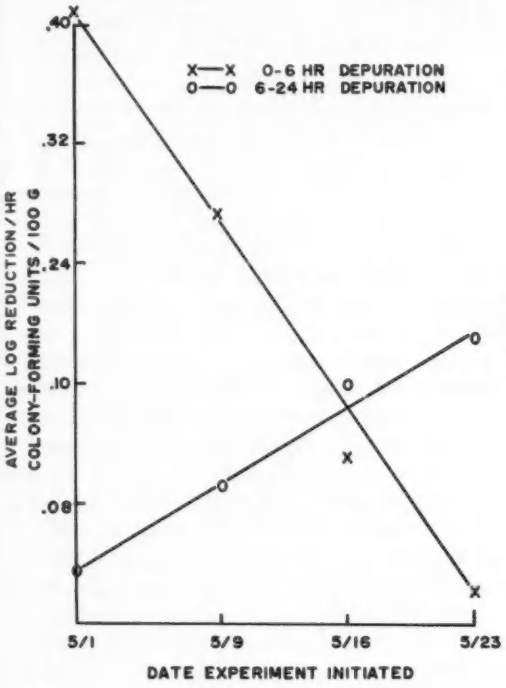

FIG. 4. Reductions in the *E. coli* content of the animals over the first 6 hr and the subsequent 18 hr of the depuration interval for each of four weekly trials performed from May 1 to 23, 1966.

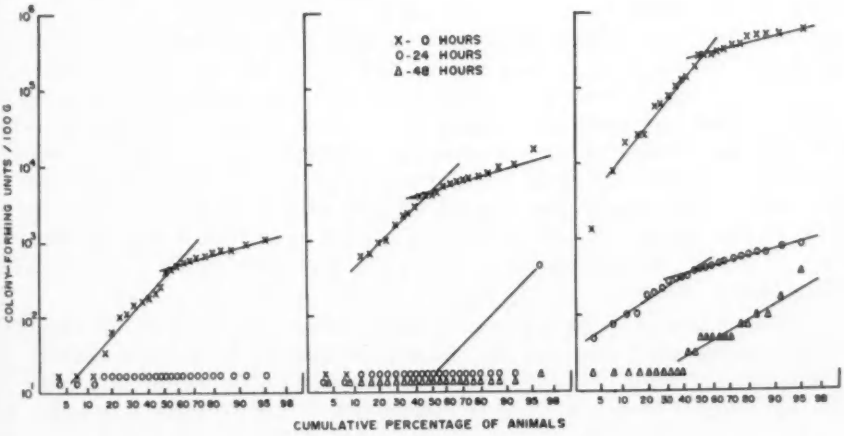

FIG. 5. Variability in the ability of individual animals to eliminate artificially introduced *E. coli* as a function of the initial contamination level. A, B, and C document trials with low, intermediate, and high initial contamination levels respectively. Each point represents the level in an individual animal. Arrows (↓) indicate levels below the sensitivity of the assay system.

accumulation appears to be more sensitive to environmental water temperatures in the 10–20 C range than does elimination.

Whereas several of the environmental parameters, such as temperature, salinity, turbidity, flow rate, contamination level, significant to the depuration process have been defined insofar as the elimination of *E. coli* by the quahaug is concerned, the effect of the season, or ambient temperature, or both, from which the animals are taken remains vague. There is a strong suggestion that animals taken during late fall, winter, and early spring from waters having temperatures below 10 C and then allowed to accumulate *E. coli* at 20 C subsequently do not eliminate the organisms as readily as animals collected during the rest of the year. This could have been due to a seasonal influence directly on the elimination processes. It also is possible that the direct effect was on the accumulation processes, in which instance decreased elimination could have been an artifact produced by allowing the quahaugs to accumulate the organisms shortly after they had been transferred from the ambient to the 20 C water. It is of interest that this "seasonal" effect on elimination was dependent on the *E. coli* level in the animals and that the "winter" quahaugs still showed a sharp, initial decrease in *E. coli* levels shortly after the elimination phase of the experiments was started. These observations and the data from the four trials in May suggest the possibility that, under the influence of "thermal shock" during uptake, some of the organisms may have been deposited at a site that is less amenable to their removal during the normal feeding processes; and it is these organisms that remain as the residual. The number of organisms so deposited would increase with increasing levels of the organisms in the water during the uptake phase of the experiments.

Since the time required to eliminate the organisms to a given level was found to be dependent on the initial level of contamination, it follows that, in any depuration process to be accomplished in a relatively short period of time — within 48 hr — there must be a ceiling on the initial contamination level. This ceiling is being determined from depuration studies with naturally polluted quahaugs and will be presented in a subsequent report. Studies on the response of these animals to chemical and physical stimuli may produce the means whereby the upper limit for the initial level of contamination may be raised, or the elimination interval may be shortened, or both. More important, the use of chemical or physical stimuli could reduce the variability in response of the animals being depurated, especially under marginal environmental conditions.

The variability in the response of individual animals must be considered in the establishment of criteria for the sanitary quality of depurated shellfish. These data are being accumulated and will be presented in a later paper dealing with target levels for bacterial depuration of the northern quahaug.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical assistance of Mr K. Lancellotti.

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Elimination of Bacteria by the Soft Shell Clam, *Mya arenaria*[1]

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CABELLI, V. J., AND W. P. HEFFERNAN. 1970. Elimination of bacteria by the soft shell clam, *Mya arenaria*. J. Fish. Res. Bd. Canada 27: 1579–1587.

Factors significant to the uptake and elimination of coliform bacteria by the soft shell clam, *Mya arenaria*, were investigated. Uptake kinetics resembling those in the northern quahaug suggest that a steady state exists in functioning animals wherein the number of organisms taken in and eliminated are in balance. In contrast to the quahaug, most of the animals accumulate the organisms very shortly after being placed in the environmental water. The lower limits on the temperature, salinity, and flow rate of the environmental water for acceptable elimination (depuration) were about 10 C, 20‰, and 7 ml/min per animal respectively. In the feasibility study for a flow-through depuration system using naturally polluted animals, consistently good elimination to very low elevated temperature (ET) coliform levels was observed if the initial level of contamination did not exceed about 2000 ET coliforms per 100 g. Results comparable with those in the flow-through system were obtained in a recirculating depuration system, thereby indicating the feasibility of the recirculating depuration system for the elimination of coliforms in marginally polluted animals.

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INTRODUCTION

FACTORS RELEVANT to the uptake and elimination (depuration) of *Escherichia coli* and elevated temperature (ET) coliforms by the northern quahaug, *Mercenaria mercenaria*, were described in previous reports from this laboratory (Cabelli and Heffernan, 1970a, b; Heffernan and Cabelli, 1970a, b). They included the kinetics of uptake and elimination, the influence of environmental parameters such as temperature, salinity, turbidity, and flow rate, the ceiling on the initial level of contamination to consistently achieve target residual levels over a 48-hr treatment period, and the variability in the response of individual animals. Some, but not all, of these factors were considered by other investigators who examined the uptake and elimination of coliforms by the soft shell clam, *Mya arenaria* (Arcisz and Kelly, 1955; Erdman and Tennant, 1956; Goggins et al., 1964). The present study was initiated to compare the uptake and elimination of *E. coli* and ET coliforms by the two species, to reexamine the influence of environmental parameters on the depuration of bacteria by the soft shell clam, and to test the feasibility of recirculating as well as flow-through depuration systems for this species, particularly as regards the existence of a

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ceiling on the initial level of contamination, target residual levels, and a means of monitoring the process.

MATERIALS AND METHODS

Soft shell clams used in this study were collected from waters in the vicinity of Revere, Saugus, Newburyport, Quincy, and Gloucester in Massachusetts and from Wickford Cove and Allen's Harbor in Rhode Island. Animals taken from the Merrimac River near Newburyport were used exclusively for the studies on the accumulation of *E. coli* and on the influence of temperature, salinity, and water flow rate on the elimination of coliforms by the clams. To study the accumulation of *E. coli* by the clams, the polluted animals first were depurated for several days to reduce their *E. coli* content to undetectable levels.

The influence of the level of *E. coli* in the environmental water and the duration of the uptake interval on the accumulation of the organisms by the clams were examined using procedures described for the quahaug (Cabelli and Heffernan, 1970a). The environmental water in the experimental depuration system was contaminated with *E. coli* suspensions as noted previously. During uptake, the water was maintained at a temperature of 16–20 C, a salinity of 28–31‰, and a flow rate of 15 ml/min per animal. At each sampling period, 24 individual animals were examined for their ET coliform levels using the elevated temperature coliform plate (ETCP) method of Cabelli and Heffernan (1970a). Because of the physical nature of the homogenates from soft shell clams, it was found necessary to distribute the 120 ml of homogenate–medium mixture into 10 petri dishes instead of the six used with water and quahaug samples.

The temperature, salinity, and flow rate of the environmental water during depuration were varied in the experimental depuration unit by the methods described previously for the quahaug (Heffernan and Cabelli, 1970a). Naturally polluted clams were used in these studies.

The feasibility of a flow-through depuration system operative under environmental conditions found appropriate in the first phase of this study (temperature, 16–20 C; salinity, 28–31‰; flow rate, 8–15 ml/min per animal) was examined using naturally polluted animals from the several locations noted above. Upon arrival at the laboratory, four pools of six animals each were assayed for their ET coliform content. The remaining animals were placed in the depuration tanks and treated for 48 hr. At the end of this period, four pools of six animals each were examined to determine the residual ET coliform levels. Twenty-four such trials were performed. During several of the early trials, fecal coliform most probable number (MPN) determinations also were performed before and after depuration to determine the comparability of results by the two methods.

A system in which the environmental water was recirculated was compared with the flow-through process for the depuration of *M. arenaria* in a series of paired trials using naturally polluted animals. The system used for recirculating the water is shown in Fig. 1. The ratio of water to clams

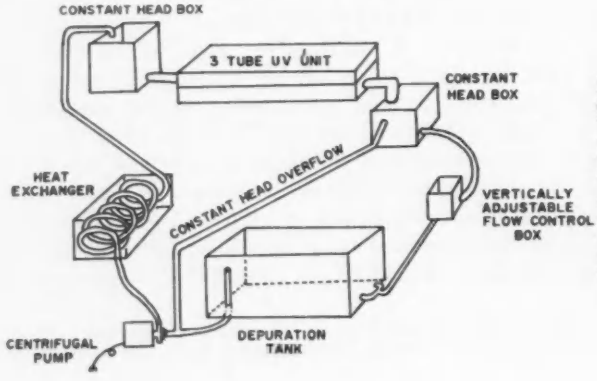

FIG. 1. Experimental recirculating depuration unit. The heat exchanger was used to dissipate the heat introduced by the ultraviolet unit and maintain the temperature at 17 C. The constant head overflow was necessary because the flow rate through the centrifugal pump could not be adjusted.

in the system was set at 95 ml/animal and the water was recirculated at a rate of 8–15 ml/min per animal. Assays for ET coliforms in the animals as noted previously were performed before and after the 48-hr treatment period.

Periodically, samples of the intake water or the recirculated water that had passed through the ultraviolet light germicidal treatment chamber were assayed to determine if they contained any viable *E. coli* cells; none were detected.

RESULTS

Accumulation of *E. coli* by the soft shell clam, *M. arenaria*, followed the same pattern observed with the northern quahaug, that is, the level achieved was determined by the *E. coli* density in the environmental water (Fig. 2A) and not by the accumulation interval after the first 6 hr (Fig. 2B). Unlike

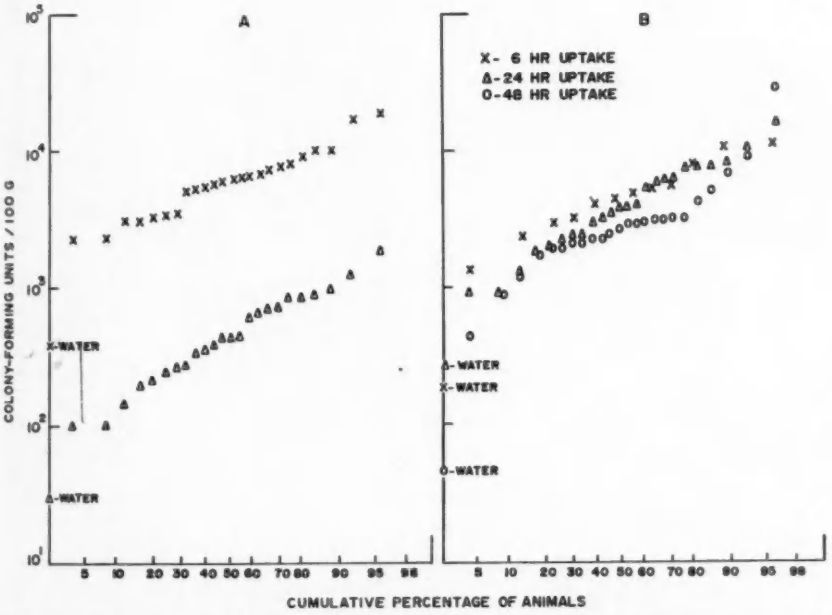

FIG. 2. Accumulation by individual soft shell clams, *Mya arenaria*, of *Escherichia coli* in the environmental water as a function of (A) the level of the organism in the water and (B) the contact time, 6, 24, and 48 hr. When the level of *E. coli* in the water was varied (A), the contact time was 24 hr. Each point represents the level in an individual animal; the level in the water is designated as such. The temperature, salinity, and flow rate of the environmental water were 20 C, 30‰, and 13 ml/min per animal respectively.

the quahaug, most of the clams accumulated *E. coli* to some extent within 6 hr; and the number of functioning animals, those that accumulated the organisms beyond the level in the water, did not change significantly thereafter. The average accumulation factor of 20 (median level in animals/level in water) was approximately three times that of the quahaug. Because the variability

in the response of the individual animals as seen from the log-probability plots was similar to that observed with the quahaugs and because of the independence of the accumulation interval on the ET coliform levels obtained, it may be assumed that this species also achieves a steady state condition in which uptake and removal of the organisms are balanced.

The influence of the temperature of the environmental water was examined late in February with clams taken from waters whose ambient temperature was less than 2 C. Of the four temperatures examined, 2, 8, 12, and 16 C, only 2 C environmental water showed a significant inhibitory effect on the removal of the organisms (Fig. 3).

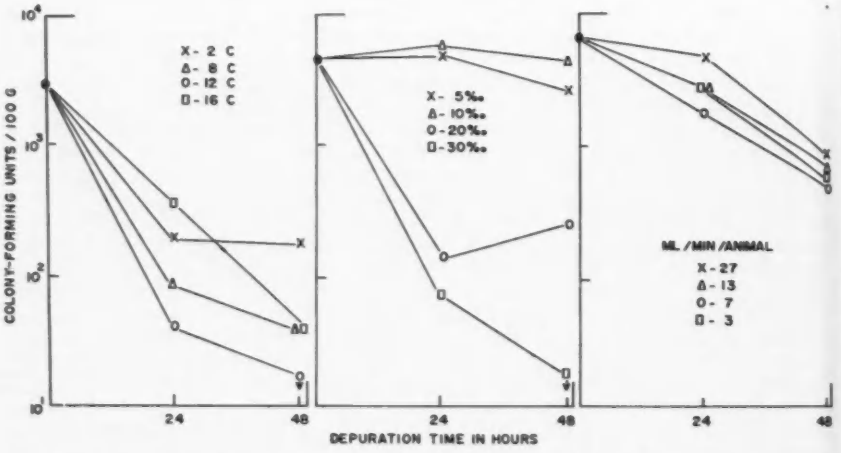

FIG. 3. Influence of the temperature, salinity, and flow-rate of the environmental water on the elimination of ET coliforms in naturally polluted soft shell clams. The temperature, salinity, and flow rate of the environmental water in the flow-through system were 16–20 C, 30‰, and 13 ml/min per animal respectively except for the parameter, which was varied.

A marked decrease in elimination activity was observed when the salinity of the environmental water was decreased from 20 to 10‰ (Fig. 3). That 20‰ is, in effect, the lower limit for good depuration activity can be seen from Table 1, which summarizes the data from two experiments in which the salinity was varied from 15 to 30‰.

No differences in the elimination of ET coliforms were observed when the flow rate of the environmental water in the depuration system was varied between 3 and 24 ml/min per animal (Fig. 3). However, at a flow rate of 3 ml/min per animal there was a significant increase in clam mortality over that in the animals treated at higher flow rates. At the lower flow rate, the siphons were observed to be markedly distended (Fig. 4); however, pumping activity, as observed visually by the emission of water, did not appear to be decreased. If anything, it appeared to be greater than in the other three groups.

TABLE 1. Influence of salinity of the environmental water on elimination of ET coliforms.

Treatment time (*hr*)	Trial no.	% residual[a] at avg salinity of			
		15‰	20‰	25‰	29‰
24	1	100	26.0	4.0	7.5
	2	36	4.7	8.9	4.7
48	1	49	4.4	1.1	6.1
	2	6.1	0.56	4.7	0.83

[a]ET coliforms.

FIG. 4. Appearance of soft shell clams, *Mya arenaria*, when the flow rate of the environmental water was restricted. (A) Flow rate restricted to 3 ml/min per animal, siphons distended; (B) flow rate, 13 ml/min per animal.

Coliform MPN, fecal coliform MPN, and ET coliform levels were compared in two trials with naturally polluted clams from Rhode Island. It can be seen from Fig. 5 that comparable densities of fecal coliforms and ET coliforms generally were obtained. Although the total coliform levels were higher than either the fecal or ET coliform levels before and after the treatment, the initial rates of elimination over the first 24 hr appeared to be similar, irrespective of which part of the coliform population was examined.

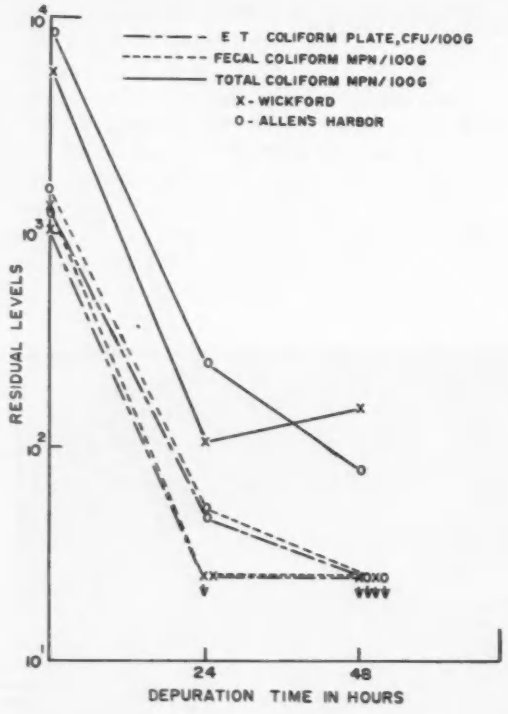

FIG. 5. A comparison of total coliform MPN, fecal coliform MPN, and ET coliform densities in naturally polluted soft shell clams undergoing depuration.

The results of a series of trials to determine the feasibility of a flow-through depuration process for the clam and the relation of the initial to residual ET coliform levels are shown graphically in Fig. 6. The ×'s are plots of the initial to residual ET coliform relations obtained in the individual trials. The ●'s are the points obtained when the trials were grouped as indicated by the boxed values and the median initial level for the group was plotted against the corresponding median residual level. Residual ET coliform levels of 25 and 50 colony-forming units (CFU)/100 g are indicated as potential targets for the process. It can be seen that with initial ET coliform levels in excess of about 5×10^3 there was no constant relation between the initial and residual levels. It can be seen that a target residual level of 25 CFU/100 g (as suggested for the quahaug in Heffernan and Cabelli, 1970b) cannot be achieved consistently if the initial ET coliform level in the animals exceeds 2000–2500 CFU/100 g.

With the flow-through data as a background, 20 recirculating trials were performed from April 22 to June 24, 1968, to determine if elimination comparable with that in the flow-through system could be obtained. In all but five of the experiments, a control, flow-through trial using animals from the same lot was paired with each recirculating trial. The initial and residual ET

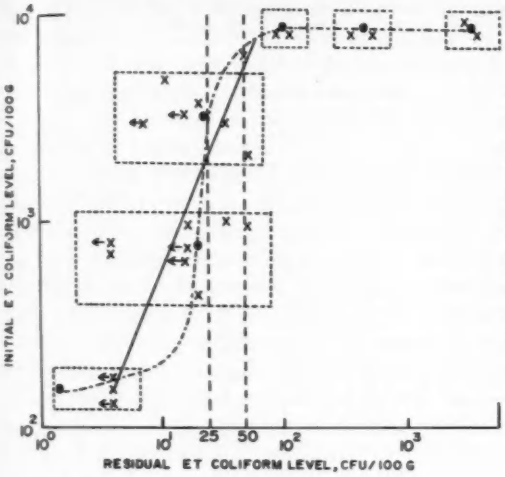

FIG. 6. Relation of the residual to the initial ET coliform levels in soft shell clams treated for 48 hr in a flow-through depuration system. Each × represents the initial and residual levels in a trial in which the densities were obtained from an examination of four pools of six animals each. The ●'s represent the average initial and residual densities for the group of trials the results of which are shown in the dotted box. The vertical lines at residual levels of 25 and 50 CFU/100 g represent possible targets for the process.

coliform levels from these trials are presented in Table 2. In trial 11, there was an equipment failure in the flow-through system. Trials 17–20 were performed in the "pilot system" (Heffernan and Cabelli, 1970b) modified to recirculate the sea water. No control flow-through system was available. There were eight trials in which the residual level exceeded the target value of 25 CFU/100 g. In three of them, the initial ET coliform levels exceeded the ceiling within which consistent depuration to the target level could be expected as seen from the previous flow-through data; high residual levels also were obtained in the two flow-through controls. The temperature of the environmental water was 20 C or more in three of those eight trials; and the water was not changed after 24 hr in the two remaining trials in which the target residual level was exceeded. In 12 of the trials, the target residual level was achieved, and the results compared well with those obtained in the control flow-through system.

DISCUSSION

The results presented herein with the soft shell clam bear a marked resemblance qualitatively to those obtained in previous studies with the northern quahaug, *Mercenaria mercenaria*; however, there are some differences in the uptake and elimination of bacteria by the two species. As reported by other workers (Kelly, 1956), the accumulation of bacteria by the clam is greater than that by the quahaug. Secondly, there is less animal-to-animal variability in the onset of activity. Depuration in the clam appears to proceed at lower temperatures than in the quahaug, and elimination to consistently low residual ET coliform levels over a 48-hr treatment interval can be achieved in the clam from higher initial levels of contamination. It is significant, nevertheless, that over a 48-hr treatment interval the same target level for the

TABLE 2. Comparison of recirculating and flow-through systems for the depuration of the soft shell clam, *Mya arenaria*.

		ET coliform level		
			Residual	
Trial	Date	Initial	Recirculated	Flow-through
1	Apr. 22	700	<4.2	4.2
2	29	520	<4.2	8.4
3		130	13	4.2
4	May 6	390	4.2	4.2
5	13	190	<4.2	<4.2
6		100	<4.2	<4.2
7	21	590	42[a]	13
8		2640	91[a]	61
9	27	680	4.2	8.4
10		6430	130	310
11	June 3	480	4.2	–
12		420	8.4	21
13	10	430	<4.2	21
14		1400	8.4	4.2
15	17	1200	240[b]	67
16		1910	30[b]	4.2
17	24	130	8.4[c]	–
18		280	26[c]	–
19		1800	50[c]	–
20		7000	67[c]	–

[a]Temperature of water 20 C.
[b]Water not changed after 24 hr.
[c]Temperatures rose to 26 C; pilot plant system used.

depurated product, 25 CFU/100 g, as determined for the quahaug is attainable with the clam if the initial ET coliform level of the animals is within the specified ceiling.

The data on the influence of the flow rate of the environmental water on the depuration activity of the clams confirms the more extensive observations made by Goggins et al. (1964). They reported that there was an adverse effect of flow rates below 1 gal/min per bushel on depuration activity. Thus, it would have been expected that over the range examined in the present study, 1.2–9.6 gal/min per bu (3–24 ml/min per animal), there should have been no significant change in elimination. That the flow rate of 3 ml/min per animal is marginal was seen from the behavior of the siphons, the marked extension of which confirms observations made by Goggins and his co-workers (1964), and from the mortality data. However, at this flow rate there is no decrease in pumping activity. If anything, it appears that pumping activity is increased, presumably because the animals attempt to increase the volume of water passed by the gills to compensate for the decrease in the oxygen content of the water in the tanks at this low flow rate. Goggins et al. (1964) reported

that about 75% of the oxygen in the water is removed at this flow rate. In this regard it is of interest to contrast the reaction of this species to a flow rate of 3 ml/min per animal to that of *M. mercenaria*. At 3 ml/min per animal, the latter species was observed to close its shell and cease pumping; there was a significant decrease in the elimination of *E. coli* from the animals (Heffernan and Cabelli, 1970a); however, there was no increase in mortality, as observed with the clam. It would appear that either the clam is much less able to cope with the effects of anaerobiosis than the quahaug, or that, in contrast to the quahaug, the continued or possibly increased activity of the clam in the presence of minimal quantities of oxygen available accelerates its death due to anoxia, or both. This could be due to a build up of CO^2 or organic acids, or both, as reported by Collip (1920, 1921), Van Dam (1935), and Dugal (1939).

In a well-aerated recirculating system in which there is sufficient environmental water for the number of animals being treated, elimination of coliforms to low residual levels can proceed as well as that observed with flow-through systems. The single most important factor that limits the widespread application of depuration for the recovery of the shellfish resource lost to pollution, as with the quahaugs, is the limitation on the initial level of contamination. Again, as with the quahaug, either a method for stimulating the animal to greater activity or a two-stage rafting-depuration system could provide an answer. Studies are in progress towards this end. However, the problem with the clam is complicated by its poor survival when it cannot burrow in the mud.

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Keeping Time of Frozen Redfish (*Sebastes marinus mentella*) Fillets in Relation to Handling of the Raw Material and Storage Temperatures after Processing and Freezing[1]

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LAUDER, J. T., W. A. MACCALLUM, AND D. R. IDLER. 1970. Keeping time of frozen redfish (*Sebastes marinus mentella*) fillets in relation to handling of the raw material and storage temperatures after processing and freezing. J. Fish. Res. Bd. Canada 27: 1589–1605.

The effect of various periods of prefreezing storage for iced, whole redfish (*Sebastes marinus mentella*) on the frozen-storage keeping times (at −23 C) of the fillets and the effect of various periods of fillet storage at temperatures equal to and higher than −23 C are reported. The fish were caught on the Grand Bank and on Banquereau in May and June. Assessment was by taste panel with chemical tests for protein solubility, rancidity, and *p*H made for purposes of comparison.

Fish iced 2 days, then processed and frozen, were of acceptable quality for 83–94 weeks; those iced for 12 days, then filleted and frozen, had a storage life to unacceptability of 51 weeks. Samples processed and frozen after 4 days in ice and subjected to variation in temperature (−23 C; −12 C) during initial frozen storage, then stored at a final temperature of −18 C, as in transportation aboard a refrigerated vessel and in cold storages used in the marketing chain, had initial quality characteristics similar to fish iced for 12 days (then processed and frozen) but had a shorter storage life to unacceptability, 44 weeks. Fish iced 15 days were unacceptable for freezing.

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INTRODUCTION

THE QUANTITY of redfish, mostly *Sebastes marinus mentella* (Sandeman, 1966), landed on the Atlantic coast of Canada in 1953, was 46,543,000 lb, valued at $1,055,000 (Anon., 1968); a preliminary estimate of the quantity landed in 1968 was 201,871,000 lb, valued at $5,250,000 (Dominion Bureau of Statistics, 1969). Because of the increased commercial importance of this species and the limited amount of technological research done on Canadian landings,

[1]The 19th in a series of papers on Newfoundland fish.

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a study was undertaken to determine the quality of redfish iced for different lengths of time (up to a maximum of 15 days) and stored under various controlled conditions after processing and freezing.

Conclusions drawn from previous studies on the quality of frozen-stored redfish are obviously based on disparate levels of quality (Appendix 1), and the observations may have resulted from different interpretations of quality as such, i.e., preference standards may have been at variance. Such nonuniformities in approach to the various researches produced results, published in Germany, Canada, Russia, and Poland, that are difficult, and in some instances impossible, to correlate. It is surprising to read widely different estimates of the frozen-storage life for this species of fish. The impression given by most European investigators is that even with careful handling before and after freezing and storage at −28 to −30 C, "maximum recommended" storage periods should not be greater than 6 months (Piskarev et al., 1961) and the fish are no longer "satisfactory" after 8–10 months storage (Partmann and Gutschmidt, 1963). On the other hand, Dyer et al. (1956) and MacCallum et al. (1967) reported that quality in frozen fillets of redfish stored at −23 C reached a level of unacceptability only after 18 and 20 months, respectively, with fish of still other catches stored commercially in 1-lb waxed paper cartons with waxed paper overwraps being considerably better than acceptable after storage for 7 months at −23 C (MacCallum et al., 1969). Seasonal and nutritional factors, reflected in the physiological condition of the catch, might account for a significant portion of the differences in the frozen-storage life of redfish, in the manner indicated by Castell (1969) for American plaice (*Hippoglossoides platessoides*). Unfortunately, the data given in the papers referred to in Appendix 1 are insufficient to permit the importance of the factors of season and state of nutrition on storage quality to be assessed; this subject will require separate investigation. More important, from the standpoint of the present work, is the fact that the available publications on redfish fail to resolve the point of greatest interest to the Newfoundland producer — the effect of the length of prefreezing storage on subsequent storage life. Partmann and Gutschmidt (1963) state that there is no difference in frozen storage quality of fish of various postmortem ages at freezing. The present work reports explicitly on this point for fish captured in late spring, including an investigation of the effect of postmortem storage in ice for 2, 4, 12, and 15 days before freezing.

EXPERIMENTAL

The redfish were from two catches obtained by the Fisheries Research Board research trawler *A. T. Cameron*. The first was obtained May 16, 1966, on the southwest slope of the Grand Bank. The fish averaged 23.1 cm (range 17–32 cm) in length, on the average slightly larger than the minimum size of 20 cm utilized commercially (Sandeman, 1966). The second catch was taken June 22, 1966, on Banquereau. These fish were of a larger size, averaging 32.5 cm (range 27–39 cm) in length.

Conforming to most commercial practices, the fish were not bled, headed, or gutted. The catches were stored in ice aboard the trawler and reiced ashore.

Initial measurements of *p*H and length were made on a representative number of scaled fish of each treatment just before hand filleting. Throughout this paper *p*H values are reported as the average, followed by the standard error. The fillets, prepared skins-on, conforming to North American market requirements, were boxed in 1-lb waxed cardboard packages.

The first catch, scaled, filleted, and boxed 14–16 fillets to a package, yielded frozen samples of three treatments: samples numbered 1, in incipient to full rigor, were packaged after 2 days in ice; samples numbered 2 were processed postrigor after 4 days in ice; and samples numbered 3, postrigor, after 15 days in ice.

The second catch, filleted and boxed six fillets to a package, also yielded frozen samples of three treatments: samples numbered 4, then in rigor, were scaled and processed after 2 days in ice; samples numbered 5 were scaled after 2 days and returned to ice storage for 10 days before being processed postrigor; and samples numbered 6 were scaled and processed postrigor after 12 days in ice.

All packages were frozen to a temperature of −23 C in 1¼ hr, enclosed in Saran Wrap[3], and stored in a master carton at −23 C for up to 90 weeks (exception, samples 2). After freezing, samples 2 were held under conditions of controlled temperature change, simulating conditions associated with transportation and storage of commercially produced fillets. This procedure consisted of storage at −23 C for a period of 4 weeks after freezing and packaging. A full master carton containing these test samples and others used for monitoring temperatures was then placed in storage at a temperature of −12 C. The fish in the centre package of the carton reached this temperature in 13 hr. After 2 weeks the fillets were lowered to −18 C (and kept at this temperature for 48 weeks), the centre package of the carton attaining −18 C within 11 hr.

Sampling of the frozen fillets was carried out as follows: first catch, samples 1 (2, 54, 90 weeks), samples 2 (9, 16, 27, 54 weeks), and samples 3 (1 week); second catch, samples 4 (4, 30, 55, 85 weeks), and samples 5 and 6 (4, 16, 30, 55 weeks).

The separate tests were done according to the following pattern:

	First catch	Second catch
Chemical assessment	On separate halves of 2–3 packages of each treatment	Mixed samples of halves of 10 packages of each treatment
Taste panel	On the second halves of packages used for chemical assessment	On separate halves of the 10 packages of each treatment

Organoleptic quality of fish may generally be defined as a combination of two qualities, taste and texture, both of which were determined by the panel. However, since redfish has a medium fat content (Hryniewiecka, 1964) and changes in this fat were expected to affect the taste, as opposed to the texture (Partmann and Gutschmidt, 1963), and since the samples became unacceptable to the panel because of low taste scores before they were rejected because of textural changes, this property was chosen as the determining factor in reporting quality. Portions sawed from the frozen package and baked, skins-on without previous thawing (Dyer and Dyer, 1949; Dyer et al., 1964b), were scored for taste and texture on a scale of 0–100. Although scores of 80–100 may be encountered in best quality fish, our experience is that top quality fillets will generally score between 80 and 85. A score below 40 for either of these attributes was considered to indicate unacceptable quality.

To promote precision in assessing the characteristics of the fish (see Table 1, 2), the panelists were usually presented with both "known" and "blind" controls (Peters et al., 1968) as well as the experimental samples. The controls were commercially prepared fillets (postmortem age at freezing not known) of various quality, scored by the panel prior to conducting the examination

[3]Product of Dow Chemical Company.

of the "test" samples. Before each of the test sittings, the panelists were told the score previously given the known control. An identically prepared pack (the blind control) was examined among the test samples as well. Results of those panelists who were unable to reconcile the scores of the blind and control samples within a span of 9.2–10.4 points were generally not used in connection with the interpretation of scores for the test samples.

Chemical tests were done on portions removed from the individual frozen packages, the portions being chopped into pieces while still frozen. The presence or absence of skins during assay is indicated in the legends to Fig. 1–6.

The samples were analyzed for extractable protein nitrogen (EPN) by the method of Dyer et al. (1950, 1964a) and analyzed for protein nitrogen by the biuret method (Snow, 1950). Protein content is expressed as percent nitrogen in the wet fish.

Extractable actomyosin was determined by the procedure of Dyer et al. (1950). Results are reported as percentage extractable actomyosin nitrogen in the wet fish tissue.

The *p*H at processing was obtained by pushing the tip of a combination glass/calomel electrode into a fresh incision just below the anterior end of the dorsal fin of the whole fish.

For frozen portions, the *p*H of an homogenate was measured, the latter being prepared by blending (at 20 C) a piece of frozen fish, including the skin, with a volume of 0.005 N sodium iodoacetate equal to twice the weight of fish (Marsh, 1952).

Total lipid in the fillets was determined by the method of Bligh and Dyer (1959) and expressed as percentage of the wet weight of the sample.

The thiobarbituric acid (TBA) test, reported in terms of absorbance (TBA value) at 538 mμ and indicating the presence (but not specific amounts) of malonaldehyde, was done on the frozen fillets by the methods described by MacLean and Castell (1964) and Castell and Boyce (1966). The absorbance readings were converted (conversion method by C. H. Castell; personal communication) to approximate amounts of malonaldehyde.

RESULTS AND DISCUSSION

Taste Panel Assessment

First catch (*Table 1*) — Sample 1, after 2 weeks frozen storage, started out at a reasonably high taste level (score 82–84), of the same order as that reported by MacCallum et al. (1967) for once-frozen fillets of freshly caught and 24-hr iced redfish, and by Partmann and Gutschmidt (1963) for frozen fillets of fish held 0–9 days in ice. The taste of the present samples dropped by a highly significant[3] amount to an intermediate grade at 54 weeks frozen storage, followed by a significant drop to an unacceptable level at 90 weeks storage. Thus, by the interpolation of these results, these samples have a cold storage life of 83 weeks at −23 C. This is essentially the same as that reported by MacCallum et al. (1967) for Saran-wrapped fillets of freshly caught and 1-day iced fish and by Dyer et al. (1956) for fillets prepared from iced trawler landings of unspecified postmortem age that, prior to packing, were individually wrapped in cellophane and then put in 5-lb waxed packages, both sets of experiments being carried out at −23 C. MacCallum et al. (1969) gave taste scores of 78 and 65 (still much higher quality than associated with the level of unacceptability) after frozen storage for 9 and 30 weeks, respectively for prerigor, twice-frozen, or 2½-day iced and once-frozen fillets of redfish (waxed paper wrapped). These values agree with scores of 77 and 67 inter-

[3]Except as otherwise indicated, statistical comparisons were made by the normal *t*-test. Differences were considered highly significant or significant when the *t* value exceeded that to be expected at the 1% and 5% levels, respectively.

TABLE 1. Taste panel assessment of fillets of redfish (first catch) in relation to pre- and postfreezing storage.

Sample[a]	Total storage time in iced and frozen storage (*weeks*)	Texture[b]	Taste[b]	*n*
3[c,d]	0	65.0±3.78	20.0±8.23	8
1[d]	2	81.7±1.31	82.9±1.05	24
2[d]	9	65.0±2.64	53.5±3.80	24
2[d]	16	73.8±2.35	56.9±3.47	16
2[e]	27	69.2±4.36	54.2±4.90	6
1[e]	54	63.0±3.74	52.0±7.18	5
2[e]	54	51.7±3.07	31.7±3.80	6
1[e]	90	48.8±2.95	37.5±2.50	8

[a]Samples 1 and 3 were packaged and frozen after 2 and 15 days in ice, respectively, then stored at −23 C. Sample 2 was packaged and frozen after 4 days in ice, then stored at −23 C for 4 weeks followed by storage at −12 C for 2 weeks. These samples were then stored at −18 C for 48 weeks.

[b]Average scores ± standard error of the mean.

[c]After having been found to be of poor quality, tasting was discontinued.

[d]Taste panels without blind controls.

[e]Taste panels with blind controls.

polated from the data in Table 1. The present paper, and the three previous Canadian papers suggest a considerably longer frozen storage life for redfish than do reports by Partmann and Gutschmidt (1963) on fillets stored in polyethylene bags, and by Piskarev et al. (1961), who make no mention of wrapping the fillets under study. The Partmann and Gutschmidt samples, frozen in February–March, did not have a satisfactory level of taste when stored beyond 26 weeks at −23 C, and Piskarev and his co-workers recommend that storage of redfish (catching date not specified) at −30 C should not exceed 6 months and at −18 C, 4 months. Apparently, preference standards might have been at variance with respect to the assessment of redfish processed in Canada or aboard European vessels; however, a more obvious factor that could account for some of the apparent variance in storage life is the different stages of deterioration reported upon. Specifically, a score of 40 represents the level of unacceptability in the Canadian reports. On the other hand, Partmann and Gutschmidt (1963) and Piskarev et al. (1961), in reporting storage life for poorest quality samples, refer to "satisfactory" quality and "maximum recommended" storage period, terms not specifically defined by the authors and perhaps not amenable to precise expression of quality in the stored sample.

Piskarev et al. (1961) did not indicate the wrap used on the stored fillets. Use of material highly permeable to oxygen and water vapour or, indeed, failure to use a wrap, could result in short storage life. On the other hand, MacCallum et al. (1969), referring to samples wrapped in waxed paper with greater permeability than the Saran Wrap used in the present work, reported taste scores of 65, even after 30 weeks storage.

In the present study, the early fluctuations in the storage temperatures of frozen commercial sample 2 were found to lower the quality at 9 weeks storage to an intermediate level, lower than the value of sample 1 (examined after 2 weeks frozen storage) by a highly significant amount. The quality of sample 2 remained without significant change up to 27 weeks storage, the fillets yielding a calculated plateau value of 54.8 ± 2.38, $n = 46$, for the period of 9–27 weeks; thereafter the score dropped to 31.7 (after 54 weeks). The average taste score of this sample after 9–27 weeks storage did not differ significantly from the value of sample 1 measured after 54 weeks storage, although the taste score for sample 2 after 54 weeks storage was lower than the score for sample 1 by a significant amount. Thus, at all corresponding times, the commercial, Saran-Wrapped sample had a lower taste panel rating than the 2-day iced fish of the same catch, the former reaching the unacceptable level about 39 weeks sooner than the fish not put through the series of temperature changes. This may be compared with a 2-month reduction in storage life to unacceptability for cod (*Gadus morhua*) fillets wrapped in waxed paper and stored initially at a higher temperature (−18 C) than the redfish, then raised to −9.4 C (as against short term storage of the redfish at −12 C), and finally stored at −18 C (Dyer et al., 1957a, b).

Basing their report on sensory examination of raw fillets of ungutted redfish, Hillig et al. (1960, 1962) previously reported fish of this species to be no longer fit for human consumption after 10–13 days iced storage. In the present work, sample 3, frozen after 15 days iced storage, was discarded after initial tasting when found to be of poor quality, the samples scoring only 20 for taste.

The samples generally were scored considerably higher for texture than for taste, with the fillets of fish held only 2 days in ice being superior after comparable frozen storage times to fillets of commercial handling.

Second catch (*Table 2*) — Frozen fillets of the 2-day iced fish (sample 4), with a taste score of 76.7 after 4 weeks storage, continued at a high level of quality, scoring 71.0 after 30 weeks (this drop is significant); thereafter the score took a highly significant drop to 53.1 after 55 weeks storage with another highly significant drop to 43.4 after 85 weeks storage. By extrapolation, storage life to unacceptability was 94 weeks compared with 83 weeks for similarly handled fish of the first catch.

An analysis of covariance was made between samples 1 and 4 and the samples were found not to be significantly different in their slopes or elevations so that the difference between storage lives found with these samples is not significant.

Sample 5, iced for 12 days, scaled after 2 days, gave a taste score, at 4 weeks storage, of 42.2, which was barely acceptable. The taste score for this sample then rose, by a highly significant amount, to 51.1 at 16 weeks storage, and maintained a score at 30 weeks storage not significantly different from the score at 16 weeks storage. It is not certain whether the rise in quality

TABLE 2. Taste panel assessment of fillets of redfish (second catch) in relation to pre- and postfreezing storage.

Sample[a]	Total storage time in iced and frozen storage (*weeks*)	Texture[b]	Taste[b]	*n*
4[c]	4	76.5±1.04	76.7±1.39	78
5[c]	4	63.5±1.20	42.2±2.32	79
6[c]	4	65.0±1.05	52.2±1.87	79
5[c]	16	61.7±1.02	51.1±2.12	79
6[c]	16	61.5±1.12	54.6±1.06	79
4[d]	30	73.9±1.28	71.0±2.06	45
5[d]	30	66.7±1.16	50.1±2.09	45
6[d]	30	67.7±1.17	57.3±1.58	46
4[d]	55	60.8±1.51	53.1±2.51	26
5[d]	55	64.0±1.36	37.7±1.72	26
6[d]	55	57.4±1.83	37.0±2.28	23
4[d]	85	45.6±1.23	43.4±1.41	32

[a]Samples 4, 5, and 6 were packaged and frozen after 2, 12, and 12 days in ice, respectively, then stored at −23 C.
[b,c,d]Same as footnotes b, d, and e in Table 1.

from 4 weeks storage to 16 weeks storage is real or whether the first sample may have been graded unduly low by comparison with an exceptionally good control. After 30 weeks frozen storage, sample 5 dropped by a highly significant amount to a score below the acceptable level at 55 weeks storage. The frozen-storage life for sample 5, interpolated from the data in the table, was approximately 51 weeks. At all corresponding storage times the scores for sample 5 were lower than those for sample 4 by a highly significant amount, confirming the shorter storage life for sample 5. Comparison of sample 5 with sample 2, using the plateau value of sample 2 for the time period of 9–27 weeks, shows that these two samples did not differ, for approximately corresponding storage times, at 3 out of 4 time points. Thus, these two samples were similar in quality over the storage period.

Frozen fillets prepared from fish iced 12 days with the scales on (sample 6) were better than acceptable (but only by a small margin, similar to commercial sample 2) at first tasting. The scores for this sample did not differ significantly from 4 to 30 weeks storage, and averaged 54.2 ± 0.91, $n = 204$. At 55 weeks storage the score for sample 6 showed a highly significant drop to 37.0, giving a storage life of approximately 51 weeks as interpolated from the data. The taste scores for sample 6 were lower than those for sample 4 (frozen after 2 days in ice) by a highly significant amount, at equivalent storage times.

Sample 6 had a storage history much like that of sample 2, the two not being significantly different either in their plateau values at approximately 9–27 weeks or in their taste scores at 55 and 54 weeks, respectively.

Because of the similarities between storage histories of sample 5 and 6, a statistical comparison was made of the taste scores. A standard *t*-test applied

between points of both sets of samples at the same storage times gave inconclusive results. An analysis of variance showed that, although the two sets of samples had the same ultimate storage life, there were significant differences in taste during the first 30 weeks of storage.

Similar to the situation with samples of the first catch, those of the second scored higher in texture than in taste. Except at the 55-week point, samples of the second catch frozen from recently iced (2 days) fish were superior in texture to those prepared from 12-day iced landings.

Chemical Assessment

*p*H before freezing

First catch — The *p*H of postrigor fish (sample 2), iced for 4 days, the shortest time in iced storage for which records of *p*H are available, averaged 6.73 ± 0.03 (range 6.40–6.95), $n = 22$. This is similar to Partmann and Gutschmidt's (1963) experience, the latter recording a range of 6.3–6.7 in frozen fillets of fish processed 4 or 5 days post-mortem. The test fish attained a *p*H of 7.12 ± 0.03 (range 6.70–7.33), $n = 22$, after 15 days storage in ice (the difference between this and the *p*H of fish iced 4 days was highly significant) at which time spoilage had proceeded to a stage that, in the opinion of the taste panel, rendered the fish unacceptable for processing and freezing.

Second catch (*Table 3*) — Sample 4, then in rigor, had an average *p*H of 7.01 ± 0.02 (range 6.80–7.16), $n = 22$, after 2 days iced storage. Readings

Table 3. *p*H of unfrozen redfish (second catch) at time of processing.

Sample[a]	Avg[b]	Range of individual values	*n*
4	7.01±0.02	6.80–7.16	22
5	6.99±0.02	6.89–7.13	22
6	6.95±0.01	6.78–7.03	22

[a]Codes are the same as for Table 2.
[b]Average values ± standard error of the mean.

were not taken again until 12 days iced storage, at which time the average *p*H was 6.95 ± 0.01, $n = 22$, and 6.99 ± 0.02, $n = 22$, for the two treatments (range 6.78–7.13).

*p*H after freezing and storage

First catch (*Table 4*) — The *p*H values of sample 1, frozen in rigor, show a significant negative trend, indicating glycolysis during storage and suggesting the absence of significant prefreezing spoilage in these samples. There are

TABLE 4. *p*H[a] of frozen fillets of redfish (first catch) stored for 55 weeks (sample 2) and 95 weeks (sample 1) at the temperature indicated.

Sample[b]	Avg[c]	Range of individual values	*n*	Slope (*pH units/week*)
1[d]		6.78–6.32	10	−0.003
2[e]	6.59±0.01	6.53–6.66	9	0

[a]*p*H of frozen fillets was done on pieces of skins-on fillets.
[b]Codes are the same as for Table 1.
[c]Average values ± standard error of the mean.
[d]Samples stored at −23 C.
[e]Samples stored as described in footnote a, Table 1.

no *p*H values for unfrozen samples to provide a direct comparison but reference may be made to the *p*H of unfrozen, 2-day iced fish of the second catch. For frozen sample 1, *p*H values for the most part fell within the range 6.5–7.0 reported by MacCallum et al. (1967) for redfish frozen before and during rigor and stored for various periods up to 90 weeks. The present range is lower than the average *p*H of 6.9 reported by Hryniewiecka (1964) for redfish frozen immediately after catching and stored for 1–3 months.

The average *p*H of commercial sample 2, frozen postrigor, was about 0.1 units below the average *p*H (6.73) of the unfrozen fish. Although significant, the difference was small, showing that glycolysis was almost complete at freezing.

Second catch (*Table 5*) — All samples in the frozen state exhibited lower *p*H than in unfrozen fish. This is consistent with data for sample 2 of the first catch and underlines the changing state of *p*H with time in frozen storage

TABLE 5. *p*H[a] of frozen fillets of redfish (second catch) stored for 55 weeks (samples 5 and 6) and 90 weeks (sample 4) at −23 C.

Sample[b]	Avg[c]	Range of individual values	*n*	Slope (*pH units/week*)
4		6.87–6.39	40	−0.0017
5		6.91–6.63	40	−0.0019
6	6.62±0.17	6.60–6.99	40	0

[a]*p*H of frozen fillets was done on pieces of skins-on fillets.
[b]Codes are the same as for Table 2.
[c]Average values ± standard error of the mean.

for fish frozen during and after rigor with respect to the *p*H of the fish before freezing. Sample 4 had average values of 6.78, 6.67, 6.66, and 6.55 after periods of frozen storage of 5, 31, 55, and 90 weeks, respectively, versus a

*p*H of 7.01 before freezing; samples 5 and 6 had average values of 6.82, 6.86, 6.81, and 6.75 (sample 5) and 6.82, 6.85, 6.76, and 6.72 (sample 6) when frozen for 5, 17, 31, and 55 weeks versus a *p*H of 6.99 (sample 5) and 6.95 (sample 6) before freezing.

The *p*H of samples 4 and 5 decreased during frozen storage, similar to the situation with sample 1 of the first catch. The decrease was surprising in the case of sample 5, as the explanation given for the decrease in *p*H for sample 1 would not be expected to apply in this instance. Sample 6 had a constant *p*H during frozen storage.

LIPIDS (TABLE 6, 7)

Redfish taken in the Newfoundland area are generally considered to be of medium fat content compared with cod and herring of low and high fat content, respectively (Hryniewiecka, 1964). There appear to be fairly wide

TABLE 6. Lipid analyses of frozen fillets of redfish (first catch).

Sample[a]	Total storage time in iced and frozen storage (*weeks*)	Lipid[b] (%)	*n*
1[c]	2	1.80±0.60	3
2[c]	10	2.09±0.31	3
2[c]	15	2.02±0.39	2
1[d]	28	3.93±0.42	2
2[d]	28	3.24±0.02	2
1[e]	55	1.54±0.00	2
2[e]	55	3.32±1.14	2
1[e]	95	2.10±0.34	3

[a]Codes are the same as for Table 1.
[b]Average values ± standard error of the mean.
[c]The fillets were skinned, then blended in the chloroform–methanol–water solution while frozen. The lipid values for these samples were not included in the average given in the text because the procedure of skinning the frozen samples could have resulted in loss of some lipid.
[d]The fillets were thawed and skinned before blending as in footnote c.
[e]Lipid analyses were done on pieces of skins-on frozen fillets.

variations in the lipid content of Newfoundland redfish with season of catch, Hryniewiecka (1964) quoting a range of values of 1.9–10% for small fish, and 3.3–8.1% for large, based on the work of Pieczatina and Leonowa (1960). Hryniewiecka's (1964) results showed no significant difference in fat composition between the two *Sebastes* types, *marinus* and *mentella*, taken on the Grand Bank in September, or between fish of different size and sex, with individual values for whole, skinned fillets of fish ranging from 3.3 to 8.4%. The same publication showed the fat component in the fillets of the September catch

TABLE 7. Lipid analyses of frozen fillets of redfish (second catch).

Sample[a]	Total storage time in iced and frozen storage (*weeks*)	Lipid[b] (%)	*n*
4[c]	5	2.45±0.12	3
5[c]	5	2.78±0.34	3
6[c]	5	2.78±1.03	3
5[c]	17	2.17±0.32	3
6[c]	17	2.94±0.43	3
4[d]	31	2.85±0.17	3
5[d]	31	3.42±0.34	3
6[d]	31	2.94±0.30	3
4[d]	55	2.50±0.01	3
5[d]	55	3.01±0.61	3
6[d]	55	2.68±0.32	3
4[d]	90	2.56±0.19	3

[a]Codes are the same as for Table 2.
[b]Average values ± standard error of the mean.
[c]The fillets were skinned, then blended in the chloroform–methanol–water solution while frozen.
[d]Lipid analyses were done on pieces of skins-on frozen fillets. These values are on the average (2.85± 0.12%, $n = 21$) similar to the average of all samples, independent of treatment (2.76± 0.12%, $n = 36$). The former, which should more truly reflect values associated with redfish fillets at time of consumption (commercial fillets are normally marketed skins-on), are reported in the text.

to be distributed unevenly throughout the fillet, being most abundant in the middle part (range 4.1–12.5%) and least so in the head part (range 1.4–5.5%). MacCallum et al. (1967) reported the fat content of skinned, whole fillets of an October catch to be 3.06%.

Thus, the values of fat for the two present batches (2.76 ± 0.33%, $n = 11$; and 2.85 ± 0.12%, $n = 21$) would appear to fit reasonably well into the seasonal picture, since redfish taken in May and June, coinciding with the spawning period (Templeman and Sandeman, 1959; Steele, 1957; Sandeman, 1966), would contain less fat than September (Hryniewiecka, 1964) and October (MacCallum et al., 1967) samples.

TBA VALUES

The TBA test indicates but does not measure rancidity directly (D. M. Bishop, personal communication). The results for the first catch (Fig. 1) show a general increase in value for samples 1 and 2 with storage time. The regression line for sample 1 has a slope of 0.0029 TBA units per week. The regression line for sample 2 has a slope of 0.0025 TBA units per week, not significantly different from the slope of the curve for the 2-day iced fish.

The absorbance value of 0.022 for samples 1 after 2 weeks frozen storage corresponds to 8.9 mg malonaldehyde per 1000 g fat, quite similar to the

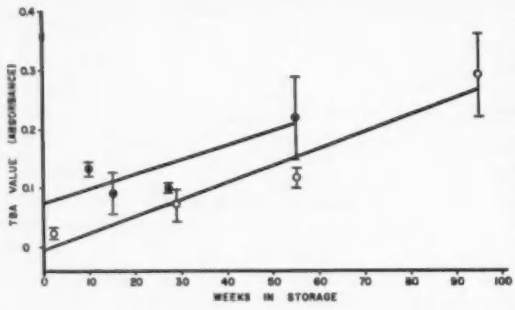

FIG. 1. Regression lines of TBA values (absorbance) obtained with frozen sample of the first catch: open circles, sample 1; solid circles, sample 2. Vertical lines centred at the points indicate the limits of two standard errors. TBA, EPN, and extractable actomyosin tests were performed on frozen samples of the first catch up to and including 28 weeks storage using pieces of fillets from which the skin had been removed while the fillets were still frozen; tests after 28 weeks were done on pieces of skins-on fillets. Statistical analysis of data for this and subsequent figures followed the system of Snedecor (1956).

value of 6.9 reported by Partmann and Gutschmidt (1963) for redfish fillets frozen after 0–9 days in ice and stored frozen for 1–2 months. The value for sample 1 interpolated from Fig. 1 after 9 months frozen storage is equivalent to approximately 46 mg malonaldehyde per 1000 g fat, about twice the value (21 mg) obtained by Partmann and Gutschmidt for frozen fillets stored for 8–9 months at −20 C.

Commercial sample 2 had significantly higher TBA values as shown by an analysis of covariance ($F = 12.80$ ($F_{0.05} = 4.07$, $F_{0.01} = 7.27$) for the absorbance–time data, Fig. 1) than the 2-day iced fish, which combined with the parallel slopes in the two instances, indicated a higher rancidity and lower quality for the commercial sample throughout the storage period, a result supporting the conclusion reached by the taste panel.

The TBA values for sample 4, the 2-day iced fish of the second catch, were all above the values for 2-day iced fish of the first catch (sample 1). The values for the two samples were significantly different at all times, based on noncoincidence of all points plotted with ±2 standard errors. Although this suggests that the iced fish of the second catch were more rancid than those of the first catch, it was not confirmed by taste panel results. Sample 4 had a TBA value of 0.125 at 5 weeks storage (Fig. 2), equivalent to 56 mg of malo-

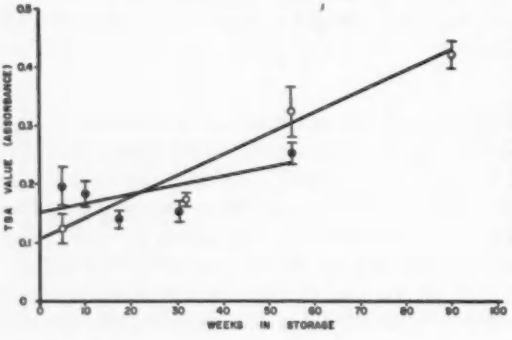

FIG. 2. Regression lines of TBA values (absorbance) obtained with frozen samples of the second catch: open circles, sample 4; solid circles, samples 5 and 6 combined. TBA, EPN, and extractable actomyosin tests were performed on frozen samples of the second catch up to and including 17 weeks storage using pieces of fillet from which the skin had been removed while the fillets were still frozen; tests after 17 weeks were done on pieces of skins-on fillets.

naldehyde per 1000 g fat, a much higher figure than obtained with fish of the first catch given similar treatment. We interpolate from Fig. 2 an absorbance value of 0.244 (equivalent to 117 mg malonaldehyde per 1000 g fat) after 39 weeks frozen storage, also considerably above the value of 0.104 (equivalent to 46 mg malonaldehyde per 1000 g fat) interpolated for the same period of storage from the data (Fig. 1) of the first catch.

As the individual TBA values for stored samples 5 and 6 were not significantly different from one another, the values for the two samples were grouped for reporting. A regression line (Fig. 2) calculated from the average values of samples 5 and 6 at 5, 10, 17, and 31 weeks storage was found not to be different from the line for the 2-day iced fish of the same catch (sample 4) in its elevation but was significantly different in slope, the 12-day iced fish having a slower rate of increase than the samples iced 2 days. The two samples were similar in TBA rating for 55 weeks storage, whereas, from the taste panel results, sample 4 was significantly better than either samples 5 or 6 at all periods of frozen storage.

Combined samples 5 and 6 and commercial sample 2 were given similar absorbance ratings after 55 weeks cold storage, agreeing with the taste panel assessment, which showed that sample 2 was quite similar to samples 5 and 6 in their storage history.

EPN AND EXTRACTABLE ACTOMYOSIN VALUES

EPN values for samples 5 and 6 were combined, as were those for extractable actomyosin, since, for each period of storage, the value of one sample fell within the range of ± 2 standard errors of the other value. All samples showed decreasing EPN with storage time (Fig. 3, 4), the slopes ranging from

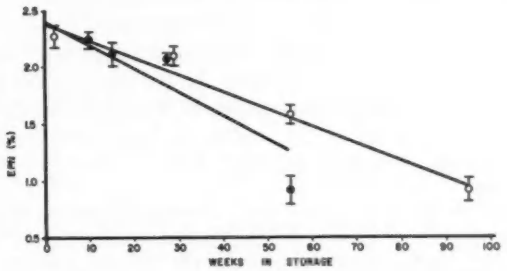

FIG. 3. Regression lines of EPN values obtained with frozen samples of the first catch: open circles, sample 1; solid circles, sample 2. Use of skinned and skins-on fillets conforms to that for Fig. 1.

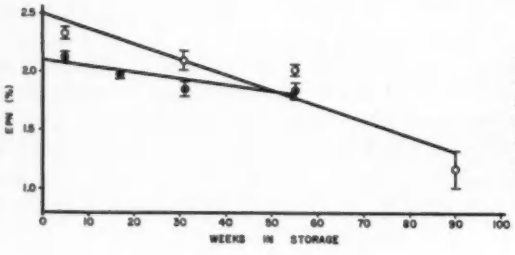

FIG. 4. Regression lines of EPN values obtained with frozen samples of the second catch: open circles, sample 4; solid circles, samples 5 and 6 combined. Use of skinned and skins-on fillets conforms to that for Fig. 2.

−0.005 (samples 5 and 6) to −0.021 (sample 2) EPN units per week of storage. Partmann and Gutschmidt (1963) reported that extractable protein (specifically myosin) did not change between 1–2 months and 8–9 months storage

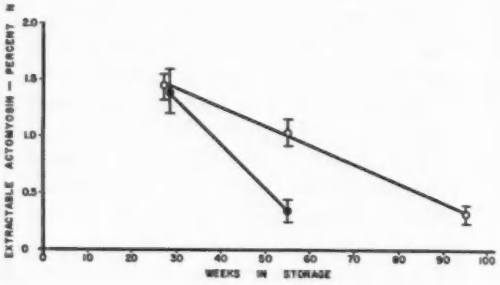

FIG. 5. Regression lines of extractable actomyosin values obtained with frozen samples of the first catch: open circles, sample 1; closed circles, sample 2. The values start with samples cold-stored for 28 weeks. Use of skinned and skins-on fillets conforms to that for Fig. 1.

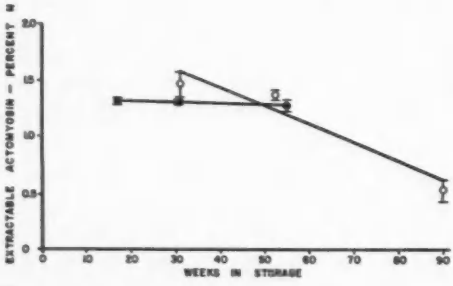

FIG. 6. Regression lines of extractable actomyosin values obtained with frozen samples of the second catch: open circles, sample 4; solid circles, samples 5 and 6 combined. The values for sample 4 start with fillets cold-stored for 31 weeks and for samples 5 and 6, for 17 weeks. Use of skinned and skins-on fillets conforms to that for Fig. 2.

at −28 C; results reported by these authors are not clear for higher storage temperatures. Dyer et al. (1956) noted that EPN values paralleled extractable actomyosin values in redfish, the latter staying steady for fish stored at −23 C over a period of 85 weeks. In the present work extractable actomyosin values show a general decrease with time in storage (as with the EPN results) with one exception — the value for combined samples 5 and 6, which remained constant during storage.

CONCLUSIONS

Apparently redfish should be iced no longer than 12 days, and the best treatment for frozen samples would be to freeze the fillets within 2 days of capture. Such short storage in ice is possible for only a portion of the catch taken during any one iced fishing trip, however. Fish iced for 12 days stayed acceptable up to 51 weeks at −23 C, a much shorter period than reported previously for once- and twice-frozen fillets prepared from recently caught raw material and for the once-frozen fillets of 2-day iced fish reported in this paper. Such shorter frozen-storage periods for fillets of fish iced for longer periods should undoubtedly be observed by segments of the industry dependent on iced landings.

Freezing fillets from fish that still retain much of their initial quality (after 4 days in ice) but subjecting them to storage temperatures that might be encountered during commercial handling and ultimately storing them at −18 C lowers the initial quality and shortens the storage life to values quite similar to those for fish that had been kept on ice for 12 days. Again, such shorter storage life, associated with commercial handling, should be observed by the processor and the wholesaler. All of the samples in this study were wrapped in Saran; storage life of frozen samples wrapped in waxed paper might be expected to be somewhat shorter, although there was no reduction from the storage life reported above as the result of the earlier Canadian work.

The average *p*H of four out of five of the redfish packs studied in the frozen state were lower than those of the fresh fish just prior to freezing and the *p*H of most of the frozen samples decreased during frozen storage.

TBA, EPN, and extractable actomyosin tests were not always in accord with taste panel results. Although these tests give some indication of condition of the frozen samples, they should not be used in place of organoleptic analysis, which, in this case, appears to give the most reliable indication of quality.

Further information on the storage life of redfish should be obtained by investigating any correlation between season of catch, initial frozen quality, and frozen storage life.

ACKNOWLEDGMENTS

We thank Dr A. W. May and Mr V. Hodder of the Fisheries Research Board of Canada Biological Station, St. John's, Nfld., for advice on the statistical methods used.

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(APPENDIX 1 *follow*)

APPENDIX 1
Data on Frozen Storage Life of Redfish (Based on Taste Panel Assessment)

Handling, and postmortem age of fish at freezing	Wrapping and postfreezing storage temp (C)	Assessed quality with months frozen storage in parentheses	Reference
Fish caught in late Feb. and early Mar. in Icelandic waters. Throats cut at capture, filleted after 0–9 days in ice	Polyethylene bagged fillets −28 (8–10)[a] −20 (5–7)[a] −12 (2½–5)[a]	"Satisfactory" level of acceptability	Partmann and Gutschmidt (1963)
Fish caught in early Oct. on the Grand Bank. Fish headed and gutted immediately after capture, filleted and in freezer within 2½ hr	Packaged in 12-oz waxed paper cartons; sealed in Saran −23 (20+)[b]	Just reaching unacceptability	MacCallum et al. (1967)
Fish headed and gutted immediately after capture, iced for 1 day, processed and frozen			
Grand Bank fish caught in Dec. Whole, unbled fish frozen prerigor, thawed after 10 days at −23, processed and fillets refrozen	Commercial 1-lb waxed paper cartons with waxed paper wrap −23	2 months[b] after capture the samples were of high sensory quality. Nearly 7 months after capture the samples were considerably better than acceptable	MacCallum et al. (1969); W. A. MacCallum (personal communication)
Unbled redfish, iced at capture, filleted after 2½ days storage, once frozen			
No data available on place, time of catch, or postmortem age of fish at time of processing. Frozen packages of fillets prepared commercially after fresh fillets had been dipped in water	Fillets individually wrapped in cellophane then packed in 5-lb waxed paper cartons −12 (7)[b] −23 (18)[b]	Just reaching unacceptability	Dyer et al. (1956)
Fish caught in May near the Labrador coast. Filleted and frozen immediately prior to development of rigor mortis	Frozen in blocks, glazed, and packed in cartons (no mention of overwraps). −25, −24, −18, and −15 (period of storage at each temperature not specified) (3)[c]	High sensory quality	Tilgner and Rogoś (1969)
Filleted and frozen after storage for full development of rigor mortis (68 hr at 14 C)	(3+)[c]	Medium sensory quality	
No data available on place or time of catch. Blocks of whole fish (?) frozen	No information on type of packing		
(1) within a few hr of catching	−30 (6)[d] −18 (4)[d] −10 (2)[d]	"Maximum recommended storage periods"	Piskarev et al. (1961)
(2) after 3–7 days storage of whole fish in ice	−30 (4)[d] −18 (3)[d] −10 (2)[d]		

[a]Assessment determined by taste and odour.
[b]Assessment determined by taste and texture.
[c]Assessment based on colour intensity, colour acceptability, texture, aroma intensity, aroma acceptability, tenderness, juiciness, flavour intensity, flavour acceptability, and overall quality.
[d]Assessment based on external appearance, taste, odour, and texture.

Maturity, Mating, and Egg Laying in the Spider Crab, *Chionoecetes opilio*

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WATSON, J. 1970. Maturity, mating, and egg laying in the spider crab, *Chionoecetes opilio*. J. Fish. Res. Bd. Canada 27: 1607–1616.

Morphometric measurements and gonad examination of the spider crab, *Chionoecetes opilio*, showed that 50% maturity occurred at about 57 and 50 mm carapace width for males and females, with minimum sizes at maturity of 51 (males) and 47 mm (females). Both sexes were mature at sizes considerably below the commercially acceptable size of 4 inches (102 mm) carapace width. After maturity, positive allometric growth was demonstrated for males. Females do not moult after reaching maturity. The small vasa deferentia found in soft-shelled mature males suggested that only hard-shelled males were capable of mating. A hard-shelled male successfully mated with a soft-shelled mature female. Field and laboratory observations suggest that more than one brood is commonly produced from one mating.

The minimum size limit for commercial landings allows some mature males capable of mating to be left on the fishing grounds. Retention of this limit seems unlikely to affect the breeding potential of the population.

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INTRODUCTION

THE FISHERY FOR THE SPIDER CRAB, *Chionoecetes opilio* (O. Fabricius), in the Canadian Atlantic Provinces has grown considerably since 1960 when the first commercial interest arose in the Gaspé area of Quebec. Up to 1966, landings of crabs caught incidentally by groundfish draggers only amounted to a few thousand pounds annually. Since 1966, exploration, stimulated principally by the Industrial Development Branch of the Department of Fisheries and Forestry of Canada, located new grounds and a trap fishery developed (Watson and Simpson, MS, 1969). Annual landings from all Atlantic provinces have increased from 1 million lb in 1967 to 18 million lb in 1969.

Although no size limit is currently enforced, processors voluntarily agreed to accept only crabs over 4 inches (102 mm) carapace width. This excluded females, all of which are smaller than 4 inches. This size limit was selected prior to any knowledge on sizes at maturity or the reproductive capabilities of the population. No information was available on the minimum sizes at which males could breed successfully or the long-term effects of fishing exclusively for males.

A limited amount of research has been conducted on *C. opilio* in Canadian waters. Brunel (MS, 1960, MS, 1961, MS, 1962) presented data on the abundance and general biology in the Gaspé area. Powles (MS, 1968) studied the biology in more detail and made preliminary estimates of male and female

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sizes at maturity. More has been published in Japan on *C. opilio elongatus*, which forms the basis of a sizable fishery in the Sea of Japan (Watson, 1969). Reproduction and sizes at maturity have been discussed by Yoshida (1941), Kato et al. (1956), and Ito (1967). Hartnoll (1963, 1968, 1969) discussed various aspects of maturity and reproduction in the Brachyura, but his studies did not include *C. opilio*.

The purpose of this paper is to discuss sizes at maturity and the breeding habits of *C. opilio* and relate the significance of the findings to the fishery.

MATERIALS AND METHODS

Crabs of both sexes were collected in the Gulf of St. Lawrence off the Gaspé peninsula at 50–130 m depth during the ice-free months May–November 1968 and 1969. A 3-m wide beam trawl lined with 13-mm mesh knotless nylon netting was used to collect small crabs. Large specimens were mostly caught in steel-framed traps 1.2 × 1.2 × .6 m covered with 75-mm mesh polypropylene netting. For maturity studies, crabs were either analysed fresh, or preserved in 10% commercial formalin for later examination. Crabs transported live on ice from the field in October 1968 were used for laboratory observations on mating and egg laying.

Size at Maturity

Morphometric measurements — Certain morphometric changes often accompany the maturity moult in the Brachyura. In females, the abdomen, pleopods, and thoracic sterna are greatly changed and in males the shape and size of the chela are often modified. The morphometric measurements shown in Fig. 1 were made using precision vernier calipers to determine whether any relations existed between morphology and maturity.

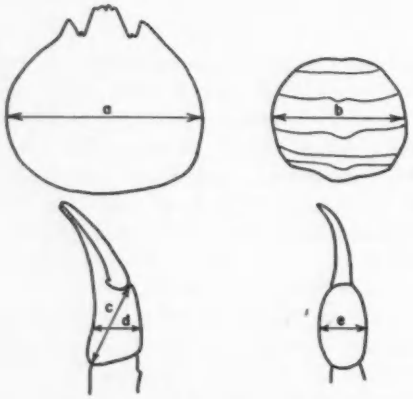

Fig. 1. Measurements used to determine the relation between morphology and size at maturity: (*a*) carapace width (♂ and ♀); (*b*) width of abdominal segment 5 (♀); (*c*, *d*, and *e*) length, breadth, and depth of palm, right chela (♂).

Gonad examination — Females (2875) (range 6–95 mm carapace width) were examined for ova and egg development. The carapace width of each animal was measured and the relative size and shape of the abdomen and pleopods were noted. Females were considered mature if they were ovigerous and had a wide abdomen with modified pleopods or, if they were nonovigerous but had the wide, mature abdomen and large, bright red ovaries that were about to be released onto the pleopods.

Wet weights of the vasa deferentia of 132 hard-shelled males (range 30–148 mm carapace width) and 62 soft-shelled males (range 54–136 mm carapace width) were determined with a Mettler model P160 balance. The vasa deferentia of each animal were catalogued according to

appearance and the occurrence of spermatophores. A further sample of 133 hard-shelled males (range 20–149 mm carapace width) was inspected for vasa deferentia appearance and spermatophore occurrence only. Males were considered mature if the vasa deferentia were convoluted and contained spermatophores.

The following four stages of vasa deferentia development were observed and are similar to those determined by Powles (MS, 1968):

(1) *Immature* — Testes and vasa deferentia not clearly differentiated. Gonads small, on either side of stomach. Vasa deferentia translucent, straight tubes; thin. Spermatophores absent.

(2) *Immature* — Testes and vasa deferentia differentiated. Testes small, coiled, on either side of stomach. Vasa deferentia translucent to opaque, straight tubes with small diverticula; thin; extend posteriorly towards heart. Spermatophores absent.

(3) *Mature*: *developing* — Testes large, coiled, spreading laterally and posterolaterally from stomach. Vasa deferentia opaque, convoluted, coiled mass up to 1.5 cm thick extends to both sides of heart. Spermatophores present.

(4) *Mature*: *ripe* — Testes similar to condition (3) but larger. Vasa deferentia opaque, convoluted, coiled mass extends to fill most of body cavity. Many spermatophores.

Mating and Egg Laying

Males and females of carapace width 55–140 and 35–80 mm respectively were held in groups or isolated in 0.3 m^3 compartments in .9 × .9 × .3-m fiber-glass tanks for mating and egg-laying studies. Each tank was supplied with aeration and filtered sea water maintained at 1–4 C and about 29‰ salinity. All animals were fed to excess on a mixture of ground cod, herring, beef liver, shrimps, and nereid worms, and tanks were cleaned daily.

To facilitate photography, mating observations were made on crabs transferred to acrylic plastic aquaria 60 × 30 × 30 cm. Egg laying was also observed and photographed in similar aquaria. To see whether new, fertile eggs would be laid in the absence of males, mature egg-bearing females were held in separate compartments until they released larvae. Fertility of newly laid eggs was established if cell division was observed.

Statistical Analysis

Morphometric data were plotted logarithmically and the regressions calculated using an IBM 1130 computer. The model $Y = aX^b$ was assumed and the lines were fitted using the logarithmic transformation $\log Y = \log a + b \log X$ where Y = carapace width in millimeters and X the variable being measured. Student's *t*-tests (Snedecor and Cochran, 1967) were used to determine significant differences in slopes between regression lines.

RESULTS

Size at Maturity

FEMALES

In the Gaspé area, 50% of females at about 50-mm carapace width are mature (Fig. 2). The smallest mature female had a carapace width of 47 mm.

Variation of the 5th (widest) abdominal segment width in relation to carapace width is shown in Fig. 3. All points on the upper line represent mature females; those on the lower line represent immature females with narrow abdomens and unmodified pleopods. The sharp break at about 50-mm carapace width indicates that morphological changes associated with maturity have occurred.

MALES

At about 57-mm carapace width, 50% of males are mature (Fig. 4). The smallest male with stage 4 vasa deferentia had a carapace width of 51 mm.

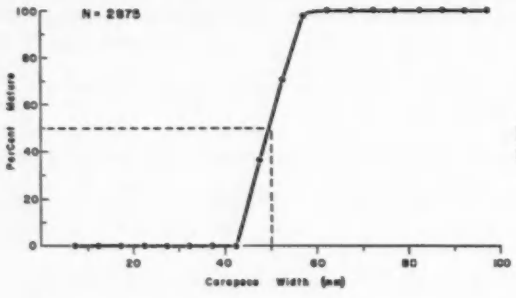

FIG. 2. Percentage maturity of females in relation to carapace width.

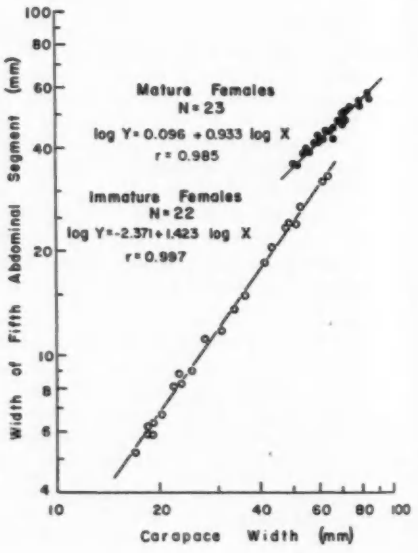
Mature Females
N = 23
log Y = 0.096 + 0.933 log X
r = 0.985
Immature Females
N = 22
log Y = -2.371 + 1.423 log X
r = 0.997
Width of Fifth Abdominal Segment (mm)
Carapace Width (mm)

FIG. 3. Relation between carapace and abdominal width for immature and mature females.

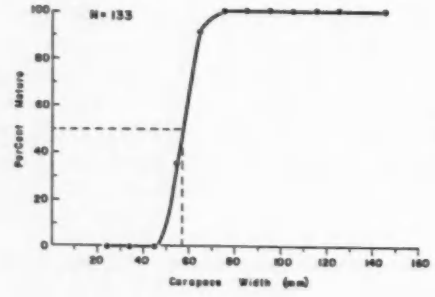

FIG. 4. Percentage maturity of males in relation to carapace width.

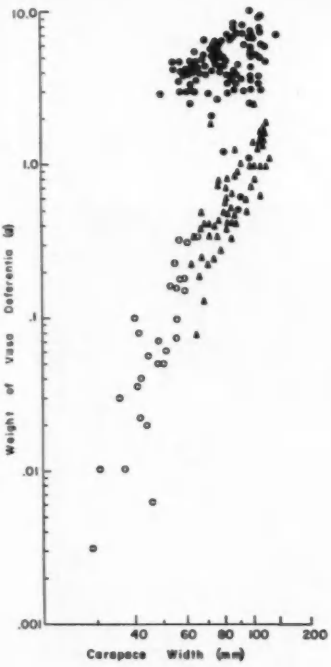

FIG. 5. Relation between carapace width and weight of the vasa deferentia. ○ = immature, hard shell; ● = mature, hard shell; ▲ = mature, soft shell.

Figure 7 shows that even in such a small animal the vasa deferentia fill most of the body cavity.

In Fig. 6 length, depth, and breadth of the palm of the right chela are plotted against carapace width. For each measurement, lines were fitted for immature and mature forms, maturity being determined by examination of the vasa deferentia. Significant differences between the slopes of the immature and mature lines were obtained for each measurement ($P < 0.001$). Clearly, the change to positive allometric growth of the chela at 55–65 mm carapace width corresponds with the onset of maturity.

Relations between weight of the vasa deferentia and carapace width and shell condition are shown in Fig. 5. Vasa deferentia of hard-shelled males showed a marked weight increase at about 55–65-mm carapace width. Those of immatures were in either stage 1 or 2, whereas all but four of the mature males had stage 4 (ripe) vasa deferentia. The four aberrant mature animals had stage 3 (developing) vasa deferentia. All soft-shelled males had vasa deferentia in stage 3 or a stage intermediate between 2 and 3, weighing 6–10 times less than those of hard-shelled males of equivalent sizes.

MATING

In the laboratory only one immature female moulted to maturity (52-mm postmoult carapace width). This animal was observed emerging from the

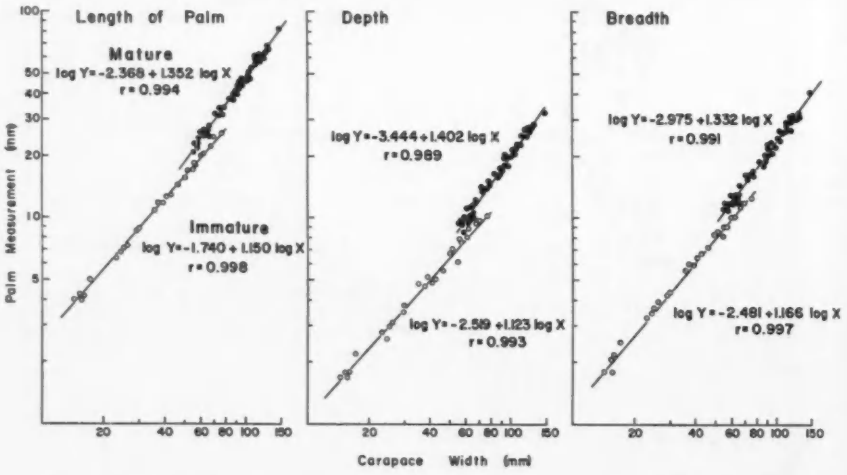

FIG. 6. Relation between carapace width and palm measurements of the male chela.

old shell on the morning of February 6, 1969. Two hours after emergence, it was transferred from its compartment, where it had been separated from males since October 1968, to the acrylic plastic aquarium. A hard-shelled male, 83-mm carapace width, was then placed with the female.

After 3 min, during which the two crabs explored their new environment, the male approached the female, walking on the tips of its legs with chela extended forwards. The female was grasped around two of its right walking legs with the left chela of the male. The female showed signs of resistance but could not escape the male's grasp. During the next 45 min the female was periodically carried around the aquarium, raised off the gravel surface above and in front of the male. When at rest the male maintained the female in this position while standing on the tips of his walking legs (Fig. 8A). Occasionally the female would be lifted almost out of the water. Periodic flexing of the male's abdomen was observed.

Gradually the female was brought down rostrum to rostrum with the male (Fig. 8B). At no time did the male lose his hold on the female's walking legs. Several attempts were made to turn the female sternum uppermost. Some resistance by the female was observed initially. At the fourth attempt, accompanied by much flexing of the abdomen, the male turned the female over so that their thoracic sterna were opposed. The female lowered her abdomen (Fig. 8C), which allowed the male to place his abdomen between the sternum and the abdomen of the female. This position (Fig. 8D) was maintained for 55 min. Spermatophores were observed issuing from the male. However, it was impossible to observe whether the male's pleopods were actually inserted into the female's genital openings.

FIG. 7. Male, 50.7 mm carapace width, with large, mature vasa deferentia.

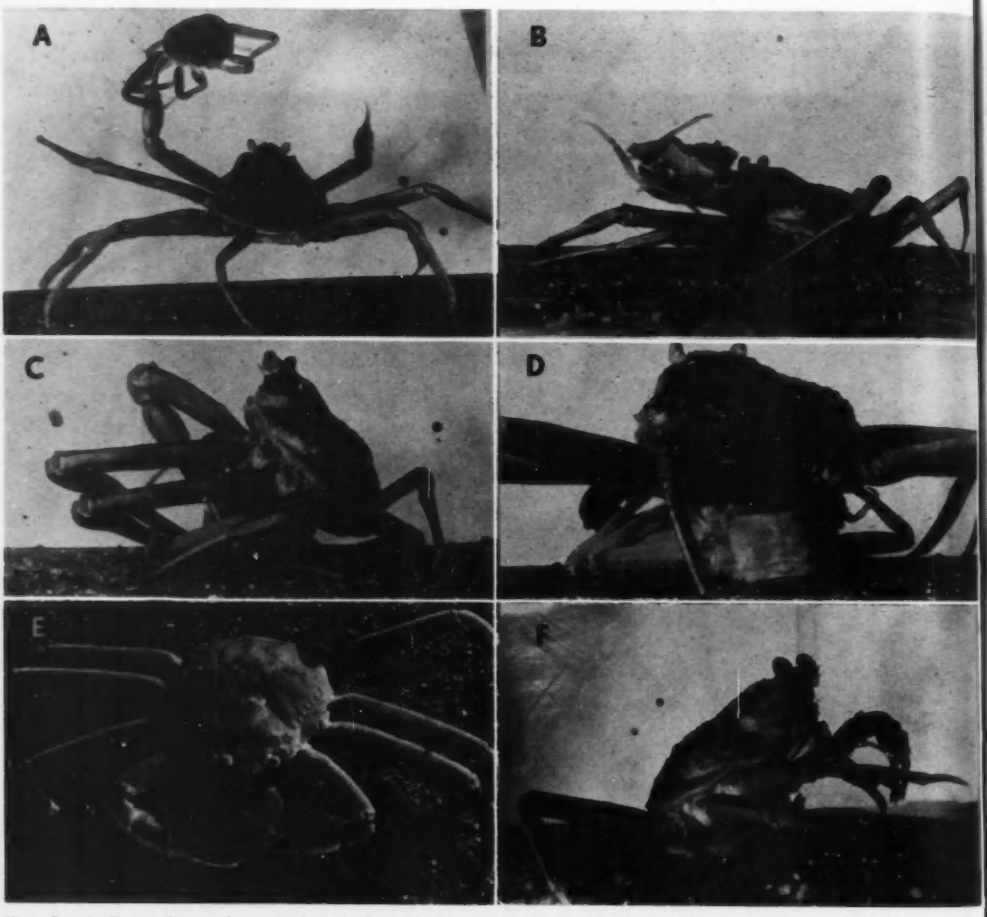

FIG. 8. A–F, mating and egg laying of *C. opilio*. Precopulatory behaviour: (A) male holds female off the aquarium floor; (B) female rostrum to rostrum with male. Copulation: (C) female turned sternum upwards. Note lowering of female abdomen; male encloses female with walking legs; (D) copulatory position. Male has inserted its abdomen between the abdomen and sternum of female. Postcopulation: (E) probable postcopulatory embrace; (F) postcopulatory egg laying.

After copulation, the male held the female close in what may have been a postmating embrace (Fig. 8E). However, 20 min after this embrace commenced, efforts to position the pair for better photographs caused the male to release his grasp on the female. Fifteen minutes later, after which time the male did not resume the embrace, the female was examined. Masses of spermatophores were seen in and around the genital openings. The female was replaced but the embrace was not resumed.

Egg Laying

The next morning, the female commenced laying eggs in small batches. Each time eggs were laid, the female rose up on the tips of its walking legs, lowered the abdomen, and released eggs onto the pleopods (Fig. 8F). When eggs ceased to issue from the genital openings the abdomen was raised and the female rested its sternum on the bottom of the aquarium. This process was repeated frequently and the egg-laying period lasted at least 4 hr, after which observations ceased. The eggs proved to be fertile.

Field observations indicated that new eggs were produced within a few days after females with old, hard shells had released larvae (J. Watson, unpublished data). Two females that had been segregated from males since October 1968 released larvae in the laboratory. One, 76-mm carapace width, hatched eggs on March 18, 1969, and produced fertile eggs 2 days later. The other, 65-mm carapace width, hatched eggs on March 17, 1969, but had not produced new eggs by May 5 when both these animals were accidentally killed. A postmortem analysis of these crabs showed that the former had small, white, spent ovaries and a considerable number of spermatophores in the spermathecum. The latter had very large, bright red, mature ovaries that completely filled all the available space under the carapace, but the spermathecum was devoid of spermatophores.

Females analyzed from 1969 field collections for occurrence of spermatophores in the spermathecum gave the following results:

	No. examined	No. with spermatophores	Shell condition
Immatures	9	0	New, hard
Matures	25	25	New, hard
	46	37	Old, hard

No immature females had spermatophores. The only mature females without spermatophores had old, hard shells and had probably spawned several times since reaching maturity.

DISCUSSION

The size of 50-mm carapace width presented here for 50% maturity of females is 5 mm lower than that estimated by Powles (MS, 1968). However, he admitted that his estimate could be high as his sampling was inadequate

for females below 62 mm. According to Yoshida (1941) female *C. opilio elongatus* matured in Korean waters at 63 mm; whereas, Kato et al. (1956) and Ito (1967) stated that the same species matured at 50–55 mm in the Sea of Japan. Females from Japanese and Canadian waters thus seem to mature at similar sizes.

Males were found to mature at slightly larger sizes than females (50% mature at 57-mm carapace width). This figure agrees with Powles' (MS, 1968) estimate for male crabs from the same area. Yoshida (1941) states that the minimum size at maturity for male *C. opilio elongatus* from Korean waters is 66-mm carapace width. This figure is much higher than the minimum size of 50 mm for *C. opilio* from Canadian waters.

It can be seen from Fig. 2 and 4 that morphometric measurements indicate different postmaturity growth patterns in males and females. In males, positive allometric growth occurs after maturity, but this is not the case for females. It is generally agreed (Kato et al., 1956; Ito, 1963; Hartnoll, 1969) that in Majidae the moult to maturity is the final one for females, whereas males continue to moult after reaching maturity. Differences in size of mature females are explained by natural variation in the size at which individuals moult to maturity.

Apart from the four aberrant specimens, all mature, hard-shelled males examined had stage 4 (ripe) vasa deferentia at all times during the sampling period. Although the mating period is restricted to the spring months (Kato et al., 1956; J. Watson, unpublished data), it seems probable that *C. opilio* males with hard shells are capable of reproduction throughout the year, similar to male *Cancer pagurus* (E. Edwards, personal communication). The vasa deferentia of mature softshelled males were markedly different from those of hard-shelled males of equivalent sizes. It seems as if development of the vasa deferentia from stage 3 to 4 proceeds during the intermoult period as the shell hardens. Hartnoll (1969) states that only hard-shelled brachyuran males are capable of reproduction and this evidence seems to support his statement.

Although the description of the mating process was based on one observation made under artificial conditions, certain comparisons can be made. As observed here for *C. opilio*, Hartnoll (1968) showed that male *Corystes cassivelaunus* often carries the female in its chela prior to copulation. Snow and Neilsen (1966) report a postmating embrace in *Cancer magister*. Although the evidence was inconclusive, a similar postmating embrace was observed for *C. opilio*. As the female had commenced moulting before observations began, a premating embrace similar to that reported by Snow and Neilsen (1966) was not observed for *C. opilio*.

Males mature at sizes considerably smaller than the current commercially acceptable size. Observations on embracing pairs of *C. opilio elongatus* caught in tangle nets (Kato et al., 1956) and *Chionoecetes bairdii* caught by scuba diving (G. C. Powell, personal communication) show that males in such pairs are often larger than the females. Butler (1960) presented evidence to suggest that in unexploited populations of *C. magister*, small mature males may be restricted

in their mating activities by competition from larger males but when the population was exploited, smaller males increased their breeding activity. The mating observation described here shows that a male, 83-mm carapace width, that is much smaller than the commercially acceptable size and larger than the majority of females, was capable of successful mating.

It has been shown that female *C. opilio* can produce more than one batch of fertile eggs from one mating. This phenomenon has also been observed for *C. bairdii* in Alaska (A. Kingsbury, personal communication) and Cheung (1968) demonstrated that multiple broods from one mating were common in the brachyuran *Menippe mercenaria*. Most ovigerous *C. opilio* females examined had spermatheca filled with spermatophores, which indicated that the production of more than one brood from a single mating is common. No successful matings of hard-shelled female *C. opilio* have been observed. Hartnoll (1969) noted that females of the Majidae do sometimes mate in the hard-shelled condition. Indeed, he observed that *Hyas coarctatus* females mated in both shell conditions. It is possible that female *C. opilio* sometimes mate in the hard-shelled condition.

If the minimum size limit for *C. opilio* in Canada remains at 4 inches (102 mm) carapace width, it would seem unlikely that the reproductive potential of the population will be affected as mature males capable of mating will be left on the grounds by the fishery. In Japan, the minimum size limit is 3 inches (75 mm) carapace width (Sinoda, 1968). Should the size limit be reduced in Canada, a situation could be reached where most of the males capable of mating were being harvested.

Several problems concerning mating that are of importance to the fishery for *C. opilio* remain to be solved. The most important of these are: whether small males can fertilize larger females, the smallest size at which males can mate successfully and whether they are capable of several matings in one season, the duration of pre- and postmating embraces, and the frequency of females mating while hard-shelled. Experiments to solve these problems are in progress.

ACKNOWLEDGMENTS

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Transferrin Polymorphism in Atlantic Salmon (*Salmo salar*)

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Three main patterns of transferrins, made up of two molecular types, were found by starch–agar electrophoresis in plasma of hatchery and wild Atlantic salmon (*Salmo salar*).

Distributions of the observed patterns from progenies of three hatchery matings agreed with expected Mendelian distributions in offspring of known parentage, implying that the bands have their origin in two codominant alleles. In nearly all samples of the wild salmon the genetic basis of transferrin variation was demonstrated by nonsignificant differences between observed and expected distributions when the Hardy–Weinberg formula was applied.

Frequencies of the Tf^A allele differed in samples from different rivers and within the same river; the Atlantic salmon forms genetically different populations. Interchange of stocks probably influenced the values of the different gene frequencies found.

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INTRODUCTION

THE PRESENCE OF transferrin, the serum protein that binds ferreous ions, in Atlantic salmon (*Salmo salar* L.) has been shown by electrophoresis and autoradiography (Møller and Naevdal, 1967), and polymorphism in the protein has been reported (Møller, 1970; Wilkins, 1969, 1970; see also de Ligny, 1969).

Transferrins have been useful for the identification of fish populations, particularly for those of cod (see Jamieson and Jonsson, 1970). Earlier investigators have indicated genetic diversity in salmon also (Nyman, 1966, 1967a, 1967b, 1970; see also de Ligny, 1969).

This report describes transferrin polymorphism in Atlantic salmon, discusses the genetic origin of the observed differences, and gives values of gene frequency in samples from different localities in eastern Canada and the United States.

MATERIAL AND METHODS

Plasma specimens sampled in 1969 were obtained from approximately 400 juvenile salmon (300 were hatchery fish) and 1600 grilse and large salmon (Table 1, Fig. 1). The hatchery fish came from three progenies, each from two parents only. Two progenies were kept together after one was marked by clipping the adipose fin; the third was kept separately. All the other specimens were from wild animals trapped in research counting fences or caught by nets for hatchery purposes. Individuals from 16 different localities ranging from Sand Hill River, Labrador, in the north, to Narraguagus River, Maine, in the south were represented in the material.

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TABLE 1. Localities from which salmon were obtained for plasma specimens, dates and methods of capture, life stages, and number of fish in 1969.

Sample	Locality	Date of capture	Gear	Life stage	No. fish
1	Sand Hill R., Labrador	July 23–29	Counting fence	Grilse	130
2	Adies Stream, Nfld.	Aug. 1	Counting fence	Grilse	112
3	Carleton, Que.	June 1–25	Trapnet	Large salmon	120
4	Restigouche R., N.B.	Sept. 2	Seine	Grilse & large salmon	120
5	Millbank, N.B.	July 24–29	Trapnet	Grilse & large salmon	59
6	Northwest Miramichi R., N.B.	May	Counting fence	Smolt	93
7	"	July 3–6	"	Grilse	117
8	"	July 17–29	"	Grilse	146
9	Southwest Miramichi R., N.B.	Sept.–Oct.	Trapnet	Grilse & large salmon	117
10	R. Philip, N.S.	July–Sept.	Fishway trap	"	120
11	Margaree R., N.S.	July–Sept.	Seine	"	95
12	Saint John R., N.B.	May–June	Fishway trap	"	105
13	"	July–Oct. 15	"	"	142
14	"	Oct. 16–31	"	"	91
15	Machias R., Maine	June–Sept.	Counting fence	"	32
16	Narraguagus R., Maine	June–Sept.	Counting fence	"	24
17	Margaree Hatchery, N.S.	Dec. 10		Parr	100
18	"	"		"	100
19	"	"		"	102

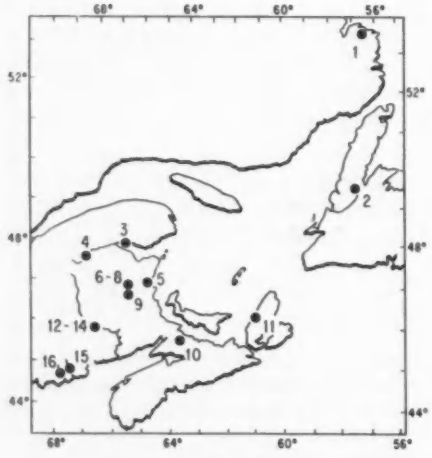

FIG. 1. Location of capture of the 16 wild samples.

The fish were anesthetized with tertiary amyl alcohol or M.S. 222-Sandoz before bleeding. Blood was drawn by cardiac puncture from grilse and large salmon and by cutting the tails of smaller fish. To prevent haemolysis, all samples were heparinized. The whole blood was kept cool

during storage and transported to the laboratory where specimens were centrifuged. The plasma was pipetted off and analysed or put in a deep freeze for subsequent analyses.

The electrophoretic technique used was a modification of starch–agar gel electrophoresis on microscope slides (Sick, 1965; Møller, 1966). The buffer giving best separation of the transferrins was a tris (hydroxymethyl) amino methane (Tris) buffer modified after Aronsson and Grönwall (1957): 60.6 g Tris, 12.8 g boric acid, and 6.0 g ethylenediaminetetraacetic acid in 1 liter distilled water.

The *p*H of the buffer varied between 8.0 and 8.2. Control of *p*H at 8.1, the preferred level, was achieved by mixing two stock solutions, one with 40 g of boric acid instead of 12.8 g and the other with no boric acid.

The microscope slides were covered by 3 ml of gel, and after application of the sera specimens the gels were covered with a thin sheet of plastic.

Each electrophoretic run contained 20 specimens, two on each slide, and lasted for 5½ hr with approximately 6 v/cm gel. After the run, the proteins were fixed in a 5:5:2 mixture of methanol, water, and glacial acetic acid, then stained with amido black 10B. The stain was then removed from the gel in the fixative and the gel was dried.

Centrifuging, pipetting specimens, and electrophoretic analysis were performed in a refrigerated room (−2 to +2 C). However, heat generation still made it necessary to have ice in the compartment underneath the slides.

Where the patterns were difficult to define, specimens were reanalysed, this time with only one specimen on a slide.

RESULTS

Patterns Found

Three common patterns of plasma proteins (Fig. 2) were found. Most of the individuals possessed a slow moving (i.e., anodic) component (1) in common. Next to this band all individuals had either one or both proteins 2 and 3. Still faster bands (4 and 5) were present in most animals.

Band 2 has been shown to bind ferreous ions (Møller and Naevdal, 1967). The difference in electrophoretic mobility between bands 2 and 3 is interpreted as phenotypic expression of codominant alleles. Three phenotypes were distinguished, Tf AA, Tf AC, and Tf CC, involving one or both of the bands Tf A (band 3) and Tf C (band 2).

Bands 1, 4, and 5 (Fig. 2) have not been identified. Both their occurrence and their intensity varied occasionally, and their presence could depend upon environmental factors. Blood samples with a high degree of haemolysis resulted in electropherograms showing haemoglobin patterns also. The haemoglobin pattern did not obscure the identification of bands 2 and 3.

Eight rare patterns in the transferrin region (Fig. 3, types 4–12) were also found. In types 4 and 5, Tf C and Tf A, respectively, are found together with a band with the same intensity as the transferrins. The band that has not been found alone had only slightly faster anodic mobility than Tf C. It is presumed to be another transferrin and it is therefore called Tf B. In routine analysis, however, it was difficult to distinguish Tf BC from Tf CC and Tf AB from Tf AC. When detected, therefore, the band was ignored and the patterns counted as Tf CC and Tf AC, respectively.

In the patterns 6–12 (Fig. 3), one or two faint bands are present together with Tf A or Tf C or both. One or these bands had a slower anodic mobility

than all the other bands found in the transferrin region; the two others had the same mobility as Tf A and Tf C, respectively. These bands were very rare. The highest number of specimens found in one sample with one of these patterns was 3 out of 100. Types 6–10 were denoted Tf AC; type 11, Tf CC; and type 12, Tf AA.

The intensity of the bands varied. As a rule the one-band patterns were stronger than the two-band patterns, and in a very few Tf AC patterns, Tf C sometimes had a more pronounced appearance than Tf A. In some rare specimens where Tf A was weak, it was impossible to decide if the pattern was a real Tf AC or type 6 in Fig. 3.

The electrophoretic patterns were always distinct when using specimens up to about 1 week old. Frozen sera usually gave reliable results over several months, but repeated freezing and thawing affected the distinctness of the bands, first the weak ones and later the major patterns.

Distribution of Patterns in Hatchery Fish

The observed distributions (Table 2) of the three patterns Tf AA, Tf AC, and Tf CC in parr of the three progenies (samples 17, 18, and 19) are different. Only the major patterns were present, and the progenies appear to represent

Table. 2. Observed distributions of the transferrin patterns in hatchery samples and distributions expected on basis of presumed genes of parents.

	Observed				Expected		
Sample	Tf AA	Tf AC	Tf CC	Probable parents	Tf^A/Tf^A (homozygotes)	Tf^A/Tf^C (heterozygotes)	Tf^C/Tf^C (homozygotes)
17	51	49		$Tf^A/Tf^A \times Tf^A/Tf^C$	50	50	
18	1	99		$Tf^A/Tf^A \times Tf^C/Tf^C$		100	
19	29	52	21	$Tf^A/Tf^C \times Tf^A/Tf^C$	25.5	51	25.5

three different crosses between the three genotypes of the two allele systems postulated above. In sample 18, however, a single individual had a Tf AA pattern, whereas all of the others had the Tf AC pattern. The parr of this sample were still available. A single fish in this sample had a clipped adipose fin, indicating that it came from a different pair of parents than the other fish; it was assumed that this individual gave the anomalous result.

The observed distributions were similar to the Mendelian distributions expected on the basis of the presumed genes of the parents (Table 2), and the two codominant alleles involved are called Tf^A and Tf^C.

Distribution of Patterns in Wild Fish and Comparison of Gene Frequencies from Different Localities

There is good agreement between observed and expected distributions in all samples of wild fish except those from the Miramichi River (samples 7

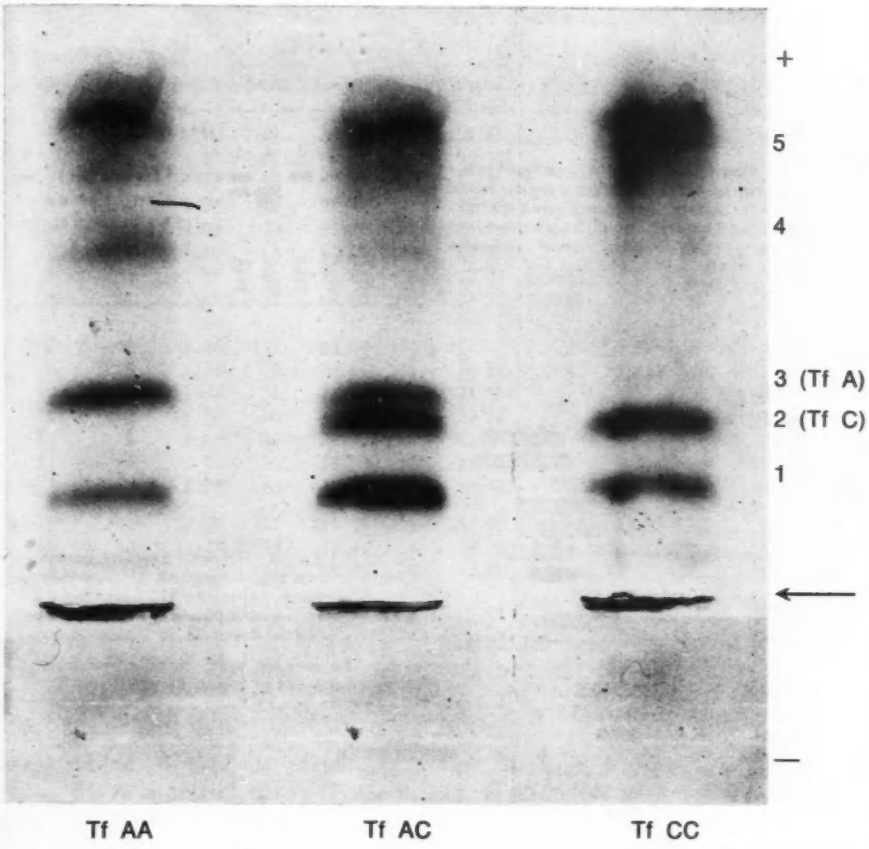

FIG. 2. Main plasma patterns found in Atlantic salmon by starch–agar electrophoresis. Bands 1, 4, and 5 are unidentified molecules; bands 2 and 3 are transferrins. Arrow indicates site of application.

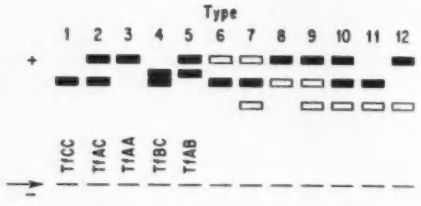

FIG. 3. Common (types 1–3) and rare (types 4–12) patterns in the transferrin region found in Atlantic salmon by starch–agar electrophoresis. Solid bars, bands with high intensity; open bars, faint bands. Arrow indicates site of application.

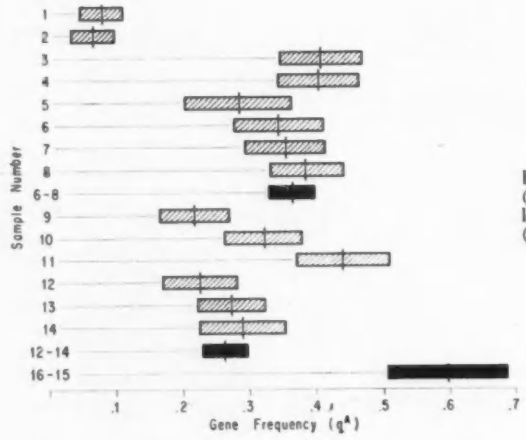

FIG. 4. Observed mean frequencies (vertical lines) and 95% confidence limits (horizontal bars) of Tf^A (q^A) in samples of wild fish. Sample areas as in Table 1.

8, and 9) and the subtotal of the samples (12, 13, and 14) from the Saint John River (Table 3). The frequency of the Tf^A allele varies greatly. The lowest value, 0.0714, is found in sample 2 from Adies Stream, Newfoundland; the

TABLE 3. Observed distributions of the transferrin patterns in the wild fish, distributions expected according to the Hardy–Weinberg law, and frequencies of the Tf^A allele (q^A).

Sample	Tf AA		Tf AC		Tf CC		χ^2	Probability of worse fit	q^A
	Obs.[a]	Exp.[a]	Obs.	Exp.	Obs.	Exp.			
1	2	0.93	18	20.14	110	108.94			.0846
2	1	0.57	14	14.85	97	96.57			.0714
3	22	20.42	55	58.16	43	41.42			.4125
4	21	20.01	56	57.98	43	42.01			.4083
5	7	4.90	20	24.20	32	29.90			.2881
6	11	11.36	43	42.29	39	39.35			.3495
7	22	15.08	40	53.84	55	48.07	7.7522	0.01–0.005	.3590
8	28	22.25	58	69.50	60	54.25	3.9983	0.05–0.025	.3904
6–8[b]	61	48.58	141	165.86	154	141.57	7.9728	0.01–0.005	.3694
9	10	5.78	32	40.44	75	70.78	5.0941	0.025–0.01	.2222
10	12	12.68	54	52.65	54	54.67			.3250
11	19	18.57	46	46.86	30	29.57			.4421
12	8	5.49	32	37.03	65	62.48			.2286
13	14	10.71	50	56.57	78	74.72			.2746
14	10	7.72	33	37.57	48	45.72			.2912
12–14[b]	32	23.70	115	131.60	191	182.70	5.3777	0.025–0.01	.2648
15	10	11.28	18	15.44	4	5.28			.5938
16	9	8.76	11	11.48	4	3.76			.6042
15–16[c]	19	20.04	29	26.92	8	9.04			.5982

[a]Obs. = observed; Exp. = expected.
[b]All three samples taken in the same locality.
[c]Samples from two neighbouring rivers.

highest value, 0.6042, is present in sample 16 from Narraguagus River, Maine. There is no indication (Table 3, Fig. 1) that this variation follows a latitudinal cline.

The frequency values of q^A (Fig. 4) make it possible to divide the samples into three different groups: samples 1 and 2 from Labrador and Newfoundland, samples 3–14 from New Brunswick and Nova Scotia, and samples 15–16 from Maine. A large area was unsampled between samples 2 and 3; the discontinuity between these two samples, therefore, is not surprising. This cannot apply to the second case, however, because the distance between the river mouths is only about 120 km and there are only about half a dozen salmon-producing streams in between.

In the second group, each of samples 6–8 differed from sample 9 in gene frequency ($P < 0.05$), and sample 11 from each of 12–14. Samples 6–8 were

all from the Northwest Miramichi, and sample 9 from the Southwest Miramichi. Sample 5, from the estuary of the river, had a value intermediate between those for the two main branches.

DISCUSSION

Interpretation of Bands

The two bands called Tf A and Tf C (either one or both were present in all Atlantic salmon investigated) are well-defined in starch-agar electrophoresis. Although Tf C has ironbinding capacity (Møller and Naevdal, 1967), this has not been shown for Tf A. The distribution of the three patterns, Tf AA, Tf AC, and Tf CC, in hatchery as well as wild fish, however, proves that Tf A and Tf C are phenotypic expressions for two codominant alleles.

It has not been possible to test the band called Tf B. Both the position and the strength conform to the transferrin bands and the protein is interpreted, therefore, as a rare transferrin component.

For expediency in classification, a Tf B always was counted as a Tf C. In other words Tf B and Tf C were treated as one single character. This treatment will not have any influence on the observed and expected distributions of the two groups of characters, Tf A being one group and Tf B and Tf C being the other. This course of action, however, could cause some loss of the capability of the transferrin system in regard to identification of populations. Mutual differences in the number of Tf B and Tf C are not registered.

Questions have been raised concerning the interpretation of the weak bands (see Fig. 3, types 6–12) (Naevdal, 1968). The bands found in the same positions as Tf A and Tf B were counted as Tf A and Tf B, respectively. This was done preliminarily because of the position and secondly because of the few numbers of individuals with a Tf AC pattern with a weak Tf A band. However, the number of individuals with weak bands were too few to have any great influence upon the distributions of the transferrin patterns and the calculated gene frequencies.

Identification of Populations

Differences in gene frequency between groups of individuals of the same species and disparities between observed and expected distributions of phenotypes as a result of an excess of homozygotes are characteristics commonly used to identify genetically different Mendelian populations.

The variation of the frequency of the Tf^A allele reflects the genetic variability in Atlantic salmon. The similarity between the observed distributions and the expected distributions of the transferrin patterns and the similarity in gene frequencies at the same locality of smolt as well as adult salmon (Table 3, samples 6, 7, 8; 12, 13, 14) and between neighbouring localities (samples 3, 4; 15, 16) support the conclusion that the statistically significant differences found represent differences between populations.

Two groups of samples, those from the Miramichi River (Table 3, samples 7, 8, 9) and the combined samples from the Saint John River (samples 12, 13, 14) showed significant differences between the observed and the expected distributions of the transferrin patterns. The material is too limited for any final conclusion, but the excess of homozygotes in these rivers may signify the existence of different populations further upstream (Saunders, 1967).

Genetic variability has been shown before in other salmonids. Brannon (1967, 1969) and Raleigh (1967; MS, 1969) experimented with progeny of sockeye salmon (*Oncorhynchus nerka*) taken from parents spawning above and below lakes. The inlet and outlet fry differed significantly in their rheotactic and phototactic responses, and in both cases the rheotactic response resulted in a greater proportion of each population finding its way to its nursery lake. The fry were kept in a controlled environment from the time of fertilization until the final tests of behaviour, so that any differences must be assumed to be genetic in origin.

The present results for Atlantic salmon are, thus, in accordance with the general view that nearly all crossbreeding species are made up of genetically distinct populations.

Two questions can be raised, however. Over the years there has been a great amount of interchange of stocks within west Atlantic salmon that could have contributed greatly to the present heterogeneity. Most of these introductions have been of progenies from a relatively few parental fish that, by chance, could have had a quite different gene frequency from the parental stock. The influence of such interchange of stocks on the genetic diversity that was found cannot be ignored.

However, interchange of stocks can hardly explain all the observed genetic diversity. No recorded interchange of fish has occurred between either Sand Hill River (sample 1) or Humber River (sample 2) in the north, and any other river. The difference in gene frequency between these rivers and the rivers further south is probably of natural origin.

Nor can the stocking explain the observed pattern of distribution. If anything, frequent introductions would break down the isolation mechanisms and lead to panmixia (Calaprice, 1969), but this does not seem to have happened. The reason could be the common occurrence of the efficient homing instinct (Carlin, 1969; Ueda et al., 1967) or some other possible premating mechanisms.

Transplanting of stocks is likely to have had some influence on gene frequency, but the degree of this influence cannot be determined by the results of the present investigation. This problem could probably be solved by introduction of progeny with known gene frequency that differs markedly from the frequency in the recipient river (Møller and Naevdal, 1968), by identification and use of other genetic systems, or by extensive sampling and analysis of transferrin patterns, particularly in localities where no introduction has taken place.

The second question raised by the present results concerns maintenance of a polymorphic character in small populations. It has been postulated that genetic drift, "random fluctuations in gene frequencies in effectively small populations," will result in variation in the gene frequency from one generation to another, tending towards the homozygous state (Dobzhansky, 1951). If the migration of salmon individuals is severely restricted between spawning localities, as the present results suggest, the number of breeding individuals must be low in most salmon populations, and homozygosity could probably be a common occurrence. From this viewpoint, the kind of genetic diversity revealed in this study is unexpected; both alleles are well represented in all samples. This probably indicates the existence of homeostasis or balanced polymorphism in the Atlantic salmon. It is difficult at this stage to draw conclusions; more material has to be analysed, and efforts to estimate the number of breeding individuals should be made.

The existence of genetic diversity in Atlantic salmon cannot be denied; the investigation confirms the existence of populations that are clearly different genetically. However, interchanges of stocks may have affected the gene frequencies of the original populations but not have led to a general breakdown of the natural isolating mechanism. Genetically distinct groups of Atlantic salmon still exist.

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Evaluation of Opercular Catheterization as a Method for Sampling Water Expired by Fish

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Experiments were carried out on rainbow trout to see if cannulae implanted at different positions on the operculum yield accurate estimates of mean expired oxygen tension. This value is frequently used to calculate utilization or ventilation volume in fish and should therefore be accurately determined.

Opercular oxygen tension (Pe_{O_2}) recorded from seven quiescent fish (383–426 g, 10–12 C) in aquaria was highly variable with respect to time and cannula placement. Expired P_{O_2} recorded from cannulae placed in the middle, upper, or lower part of the posterior margin of the operculum ranged from 54 to 157 mm Hg and was sometimes identical with inspired P_{O_2}. When the fish were chased, expired oxygen tension usually rose but the magnitude of the rise and its duration were not uniform from cannula placement to placement. Other studies, on 21 trout (217–388 g, 10–12.5 C) whose gills were perfused with water at different flow rates with a mouth tube, indicated that the Pe_{O_2} variability existed over a wide range of perfusion rates. In addition, Pe_{O_2} levels from all three placements at perfusion rates approximating those of normal ventilation were frequently below the mean expired oxygen tension calculated from oxygen uptake and gill waterflow rate data.

It is concluded that opercular cannulae provide poor estimates of mean expired oxygen tension in trout. The use of this technique to determine mean Pe_{O_2} could lead to serious errors. It appears that large changes in oxygen utilization can be detected with opercular cannulae but any utilization values determined in this way would likely be erroneous.

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INTRODUCTION

ONE OF THE MOST PRODUCTIVE TECHNIQUES in the study of fish respiration and circulation involves placement of glass or plastic cannulae on the fish so that both blood and water entering and leaving the gills can be sampled.

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Many workers have used variations on this approach (Van Dam, MS, 1938; Holeton and Randall, 1967; Randall et al., 1967; Saunders, 1961; Smith and Bell, 1964; Stevens and Randall, 1967).

Preliminary studies indicated that efferent water oxygen tensions recorded from single cannulae placed midway down the posterior margin of rainbow trout (*Salmo gairdneri*) opercula were quite variable. A common practice has been to calculate values such as utilization and ventilation volume from mean expired oxygen tensions measured from cleithral or opercular cannulae (Saunders, 1961, 1962; Holeton and Randall, 1967; Stevens and Randall, 1967). If mean expired oxygen tension was incorrectly estimated by these cannulation techniques such calculated values would be in error. Indeed, considerable data obtained by such techniques have been used in theoretical analysis of the gas exchange process in fish (Jones et al., 1970; Taylor et al., 1969). Thus it seemed vital to test whether such cannulae provide accurate estimates of mean expired oxygen tension and to see if cannula position influenced the results.

Extraction of oxygen from the water would be influenced by the pattern of water and blood flow through the gill sieve. Accordingly, a second aim of this study was to see how the rate of water flow across the gills affected expired water oxygen tensions.

METHODS

Experiments were carried out during 1969 on rainbow trout, *Salmo gairdneri*, purchased from the Sun Valley Trout Farm, Coquitlam, B.C. The fish were held in 250-gal tanks prior to use, and were fed a mixed diet of chopped horse heart and trout pellets three times a week. Research space was provided by the Vancouver Public Aquarium, Vancouver, B.C.

Cannulation Procedures

Small plastic cannulae were attached to the operculum of trout held under anesthesia (1:10,000 M.S. 222) on an operating table similar to that described by Smith and Bell (1964, 1967). Cannulation involved placing lengths of Clay-Adams PE 60 polyethylene tubing on the operculum so that efferent water leaving the gills could be sampled. The operculum was punctured with the tip of a #15 needle and a 3-mm length of PE 200 tubing, heat flared on one end, was passed through the operculum from inside. A length of PE 60 tubing (70 cm for experiments in aquaria, 24 cm for gill perfusion experiments) was pushed into the PE 200 sleeve from outside the operculum and held in place by a tight ligature that constricted the sleeve upon the cannula (Fig. 1). In addition to the opercular cannulae, a buccal cannula (Saunders, 1961) was positioned in fish used for experiments in aquaria. Three opercular cannula placements were tested and were

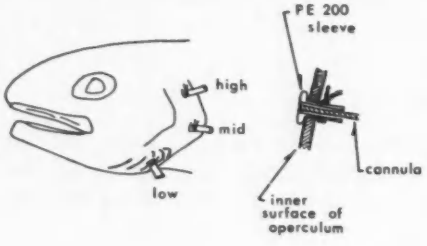

FIG. 1. Head of a trout, shown with the buccal and opercular cannulae in place. The method of attaching the cannulae to the operculum is illustrated.

termed "high," "mid," and "low" because of their position relative to the dorsoventral axis of the fish. The high opercular cannula was positioned immediately adjacent to the anterior end of the lateral line, whereas the midopercular cannula was located halfway down the dorsoventral axis of the operculum. The low opercular cannula was inserted through a hole in the 4th branchiostegal ray (counting from the dorsal-most ray in a ventral direction). All the cannulae were placed so that they penetrated the operculum about 5 mm from its posterior margin. Occasionally all three cannulae were attached to the right operculum of a single fish although usually only one or two cannulae were employed. In some instances cannulae were placed on both sides of the animal's head.

Another approach involved attachment of cannulae to the cleithrum of trout or coho salmon. Some cleithral cannulae were straight tubes that passed posteriorly through the cleithrum whereas others consisted of perforated tubes contoured to fit the cleithrum. The straight tube cannula frequently became plugged with gill filaments and it was likely that similar clogging of the contoured cannula occurred. Also, there was some doubt as to whether the contoured cannula would sample water uniformly along the dorsoventral margin of the gills. Consequently, cleithral cannulation appeared to be an unsatisfactory technique and was abandoned after preliminary trials.

Experiments in Aquaria

To study opercular P_{O_2} levels in free-swimming fish, cannulated animals were placed in covered, darkened aquaria and were allowed at least 24 hr to recover from the operation. Such a recovery period is essential since cannulation procedures involve considerable trauma (Houston et al., 1969). Experiments consisted of continuously monitoring inspired and expired oxygen tensions in water from the various cannulae for approximately an hour while the fish remained undisturbed in the darkened aquarium. The animal was then chased with a stick for a few minutes and P_{O_2} levels were then recorded for 2–3 hours following activity. Seven fish ranging in weight from 382.5 to 426 g were studied in this manner. Temperature was constant during any one experiment and ranged from 9.6 to 12 C over the series of experiments.

Oxygen tensions were measured by gently lifting the cannula tips over the edge of the tank and allowing a flow of water to drip from the tips. The cannulae did not appear to be siphoning off water as the flow was intermittent and in time with pressure pulses that occurred in the branchial chamber of the fish as it breathed. The cannula tips were periodically connected to a thermostatted cell containing a Radiometer model E54046 oxygen electrode. This electrode was attached to a Radiometer model 27 GM *p*H meter and gas monitor type PHA 928. The cell was maintained at the same temperature as the test aquarium and calibration of the system was frequently checked by exposing the electrode to water samples equilibrated to a known P_{O_2}.

Artificial Gill Perfusion Studies

An artificial gill perfusion system was employed to see if variability in expired oxygen tension was influenced by the rate of water flowing over the gills. Anesthetized trout were cannulated as previously described and a 4-cm length of stiff Tygon tubing, 9 mm I.D., 12 mm O.D., was tied securely into the mouth so that it penetrated about 1 cm into the buccal cavity. The tube was used to direct a flow of water over the gills in a plane parallel with the roof of the mouth. Once tied in place, the mouth tube eliminated any activity of the buccal pump but the opercular apparatus was free to move.

The cannulated fish was then placed in a lucite box similar to the constant flow system described by Fry (1957). The trout was held upright by lucite pegs pushed into holes in the bottom of the box in a "peg-board" fashion. The top was sealed with a wing-nut closure and the mouth tube passed out through rubber stoppers (Fig. 2).

The fish was allowed 24 hr recovery and then various water flows were passed over the gills for a period of 1 hr at a given rate. At the end of that hour Pi_{O_2} and Pe_{O_2} were recorded and the flow was reset to a new level (Pi_{O_2} and Pe_{O_2} are the oxygen tensions in millimeters Hg in inspired and expired water respectively). Flows ranged from 80 to 1200 ml/min and were applied at random except that the lowest flows were not used until the end of an experiment. Oxygen uptake was calculated at each perfusion velocity by the Fick principle from the difference in oxygen tension

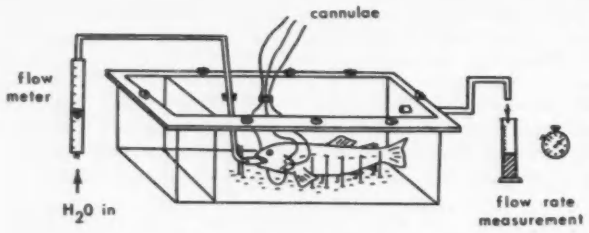

FIG. 2. A constant-flow apparatus for artificial perfusion of the gills at various flow rates. The fish is held upright by pegs pushed into holes in the bottom of the box. Cannulae pass through needles inserted in rubber stoppers in the box top.

between box inflow and box outflow water, the flow rate, and the oxygen solubility coefficient. In these experiments 21 fish ranging from 217–388 g were studied at temperatures ranging between 10 and 12.5 C.

RESULTS

Oxygen tensions in opercular water samples from quiescent trout in aquaria were extremely variable. Tension recorded from any one cannula placement in a single fish varied with time and often changed abruptly (Fig. 3A). There were large differences in tension between the three placements (Fig. 3B, C) and no one placement gave consistently high or low P_{O_2}'s. Extended observations of the same individual (cf. Fig. 3A and B) indicated that the degree of variability of any one sampling site differed from day to day. Often separate cannulae placed in the same position on either side of the fish gave results that differed by as much as 100 mm P_{O_2}.

Chasing the fish usually resulted in a rise in opercular P_{O_2} regardless of cannula position. Some placements showed a greater P_{O_2} rise following activity than others. For example, in Fig. 3B the oxygen tension in water from the midopercular placement rose to a level approaching that of inspired water, whereas tension from the high opercular placement rose only slightly. Thus, although there was always an apparent decrease in utilization of oxygen from the inspired water after activity, the magnitude of this decrease and its duration were not uniform from placement to placement.

All seven fish studied in aquaria showed similar variability in expired oxygen tensions with Pe_{O_2} levels varying from 54 to 157 mm Hg.

Studies in which the gills were perfused with water at different flow rates showed similar results. Large variability in efferent P_{O_2} was present in all 21 fish studied and this variability extended over the whole flow range examined. There was a tendency for Pe_{O_2} levels to be higher at high gill water-flow rates in all three opercular placements studied (Fig. 4). The variability in efferent P_{O_2} for all three placements is reflected by the large standard errors surrounding each point in Fig. 4.

Mean oxygen uptake of perfused fish, calculated by the Fick principle, rose from 52.7 to 104.9 mg/kg per hr as the flow rose from 0.3 to 3.75 ml/min per g body weight. Mean expired oxygen tension for the group, calculated from the oxygen uptake rate and rate of water flow over the gills, rose from

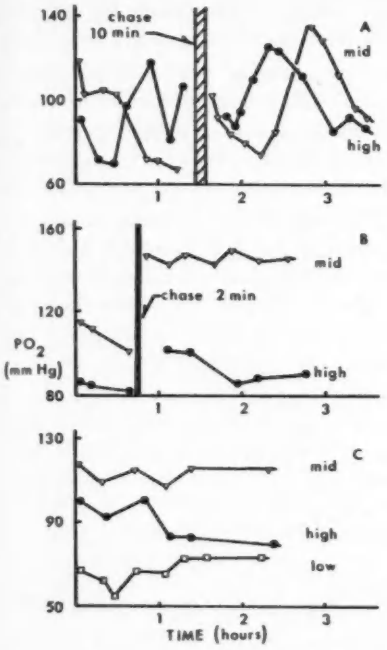

FIG. 3. Oxygen tensions recorded from free-swimming fish in covered, darkened aquaria. The three opercular cannula placements are termed high, mid, or low as in Fig. 1. Inspired oxygen tensions remained between 155 and 160 mm Hg. A and B represent two experiments carried out on a single 332.5-g trout with a 48-hr interval between experiments; C is for a 342-g fish.

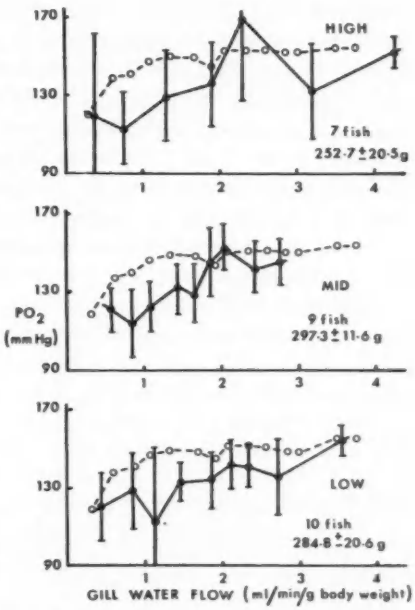

FIG. 4. A comparison of Pe_{O_2} levels measured from the three cannula placements and the calculated mean expired oxygen tension for perfused fish. The calculated values are shown as dotted lines and open circles and are based upon data for the entire group of 21 fish. Data from individual placements are shown as solid lines and solid circles and the points are means ±2 standard errors.

118 mm Hg at the lowest velocity applied to a high of 154 mm Hg at the highest flow rate. Thus calculated mean P_{O_2} could be compared with P_{O_2}'s measured from the three placements. Figure 4 illustrates this comparison and shows that at the lower perfusion rates, calculated mean P_{O_2} was often considerably higher than P_{O_2}'s measured from opercular cannulae.

DISCUSSION

Results indicate that highly variable oxygen tensions were obtained from cannulae placed at different positions on the operculae of rainbow trout in aquaria. No cannula placement appeared subject to less variation than any other. The technique did indicate that expired P_{O_2} rose when the fish was chased round the aquarium, but the magnitude of the P_{O_2} increase was not consistent from one cannula placement to another.

Experiments in which the gills were artificially perfused at different flow rates showed that P_{O_2}'s recorded from all three opercular cannula placements were quite variable over a wide range of gill water flows. At the highest perfusion rates the P_{O_2} from each of the three placements was often identical with the inspired oxygen tension. Of course utilization was taking place at these velocities, but the sampling technique failed to detect it. At perfusion velocities below 2 ml/min per g (approx 550 ml/min), P_{O_2}'s from all three placements were often below the calculated mean expired oxygen tension. Since ventilation volume in 200-g *Salmo gairdneri* at 9 C ranges from 22 to 276 ml/min (Davis and Cameron, 1970), it appears that opercular catheterization yields low P_{O_2}'s at perfusion velocities within the flow range that accompanies normal ventilation.

The high variability of opercular P_{O_2} and the disparity between the measured tensions and the calculated mean expired tension indicate that opercular cannulae provide poor estimates of mean expired oxygen tension in trout. Although it is possible to detect large changes in utilization with this technique the accuracy of measuring utilization in this way is questionable. There is evidence that this phenomenon is not peculiar to rainbow trout. Garey (MS, 1967) reported opercular P_{O_2} variability in the carp, *Cyprinus carpio*, and initial experiments in our laboratory on a 360-g starry flounder, *Platichthys stellatus*, also indicate a considerable variability in its expired oxygen tensions.

Clearly, any calculations based upon data collected from opercular cannulae could be subject to considerable error. For example, Stevens and Randall (1967) reported that Pe_{O_2} did not change from resting levels during moderate swimming activity in rainbow trout. This result was based on data from a single midopercular cannula and is likely erroneous, since Pe_{O_2} would be expected to increase when ventilatory flow goes up during activity (Van Dam, MS, 1938).

It would appear that cannulae placed through the cleithrum (Saunders, 1961) also yield inaccurate results. Saunders (1962), as pointed out by Holeton (MS, 1966), calculated a ventilation volume of 143 ml/sec for a sucker, *Catostomus commersoni*, weighing less than 250 g. This calculation was based on measures of Pe_{O_2} taken from a single cleithral cannula. Even if the ventilation rate of this fish were 100 mouth closures/min, it would have to propel 85.8 ml/mouth closure over its gills — a considerable feat, since the volume of the branchial cavity of this species would be considerably less than 86 ml. Indeed, K. Watters (unpublished data), in preliminary experiments, has shown that cleithral cannulae in trout and salmon yield Pe_{O_2}'s that are equally as variable as those from opercular cannulae.

There are a number of possible explanations for the observed variability in Pe_{O_2} levels. First, the presence of cannulae on the operculae could prevent proper movement of these structures and thereby interfere with the normal

pumping of water over the gills. If the operculae were held closed by the cannulae, then water would tend to remain stagnant in the gills and low Pe_{O_2}'s would result. If the cannulae held the operculae open, and prevented proper closure of the opercular valve, then residence time of the water in the gills would be reduced and water might tend to "leak" into the opercular cavity through the opercular opening. Under the latter conditions the opercular P_{O_2} levels could be high.

Other possible explanations for varied Pe_{O_2}'s could relate to the movement of blood and water through the gill sieve. Variations in the pattern or velocity of water flow across the gills could occur from time to time and cause resultant changes in utilization (Hughes, 1964, 1966). Indeed, these changes could be very rapid and produce alterations in utilization within a single breathing cycle.

Changeable water-flow patterns could result from adjustments in activity of the ventilatory muscles or alterations in the position of the gills relative to the water flow. Different muscles operate in shallow ventilation as compared with deep ventilation (Ballintijn and Hughes, 1965) and delicate changes in the activity of these various muscles could alter the pattern of water flow over the gills. In addition, Pasztor and Kleerekoper (1962) have reported that the adductor muscles between adjacent gill hemibranchs act to change the position of the gill filaments during different phases of the respiratory cycle. They also observed that at high ventilatory flows these same muscles act to move adjacent gill filament tips apart and allow nonrespiratory spillage of water past the gills. They suggested that this spillage is advantageous, as it prevents damage of the gill lamellae at high-water flows. Such damage, however, is extremely unlikely as the differential pressure across the gills is only 2–3 cm H_2O (Hughes and Shelton, 1958) and would therefore not be sufficient to damage the lamellae. It is more likely that the gill adductor muscles regulate the supply of water reaching the gill lamellae and keep the gill apparatus properly oriented to the water flow as Pasztor and Kleerekoper have also suggested. In addition, such adjustments could serve to alter gill resistance or keep it at a constant level to reduce form drag when a fish swims. Such muscular adjustments could certainly affect utilization of oxygen from the inspired water by changing the orientation of the gill sieve to the water flowing past it.

Variation in blood distribution to any one region of the gills could result in changeable Pe_{O_2} tensions in that region. Catecholamines are known to increase the blood flow through respiratory portions of the eel and trout gill (Steen and Kryusse, 1964; Richards and Fromm, 1969). Circulating catecholamines (Nakano and Tomlinson, 1967), or those released locally, could act on α-adrenergic receptors, which are found in gills of trout (Randall and Stevens, 1967), and cause increases in exchange area. Adjustment of blood flow to various parts of the gill could provide a means of varying oxygen extraction at any one site and would result in variable Pe_{O_2}'s at that site.

It is interesting to note that G. F. Holeton and J. Skidmore (personal communication) observed dye accumulation in only the first few gill lamellae

located adjacent to the afferent branchial artery of an isolated trout gill. They also showed that more lamellae became filled with dye in the presence of adrenalin. Such experiments suggest that the entire exchange area of the gill may not always be supplied with blood and that certain portions of the gill tissue may be "shut off" or "turned on" as required.

The best way to get an accurate measure of the mean Pe_{O_2} is to collect all water leaving the gills and thereby obtain a mixed sample of efferent water. This can be accomplished using bags or tubes attached to the fish and works well with species such as the pufferfish (Hall, 1931), dogfish (Baumgarten-Schumann and Piiper, 1968; Hanson and Johansen, 1970), and dragonet, *Callionymus lyra* (Hughes and Umezawa, 1968a, b), owing to the particular anatomy of these species.

Such procedures are far more difficult in trout however, owing to problems of interference with the proper function of the ventilatory musculature. Thin rubber or plastic membranes attached to the operculae of trout appear to load the respiratory apparatus. In several experiments conducted in our laboratory, animals have died from apparent exhaustion and inability to move sufficient water over the gills when such bags were attached. Death occured even though great care was taken to minimize interference with the ventilatory muscles and keep the resistance of the collecting bag as low as possible.

Similarly, thin rubber menbranes such as those used by Van Dam (MS, 1938) must seriously interfere with ventilation. These devices are stretched over the head to separate inspired and expired water. Such structures must constrict the branchial apparatus and prevent normal pumping.

Perhaps the most promising technique, other than choosing a more suitable species of fish, is that of tying thin rubber or plastic membranes to the mouth of the trout and leaving the delicate operculae unencumbered. Such membranes separate inspired and expired water and allow collection of a mixed sample of the latter. Experiments recently completed in our laboratory indicate that such devices do not interfere with ventilation when properly affixed (Davis and Cameron, 1970). Oral membranes appear to be preferable to techniques that load the opercular apparatus, and are certainly better than the unrepresentative results yielded by opercular catheterization.

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Analysis of Ram Ventilation of Fish Gills with Application to Skipjack Tuna (*Katsuwonus pelamis*)[1]

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Some species of fish use "ram ventilation" to pass water over the gills while swimming. Hydrodynamic analysis provides estimates of the pressure losses as the water passes through the gill spaces, and of the resistance force that must be overcome by the swimming muscles. For a 44-cm skipjack tuna (*Katsuwonus pelamis*), swimming at its basal speed of 66 cm/sec, the gill resistance is estimated to be 1090 dynes or 7% of the total swimming resistance. If oxygen consumption doubles, gill resistance increases to 27% of the total. The energetic cost of respiration lies between 1 and 3% of the total metabolism at basal swimming speed.

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INTRODUCTION

THE ACTION of the buccal and opercular pumps in forcing water over the respiratory surface of fish gills has received considerable attention (e.g., Hughes, 1960; Hughes and Shelton, 1958; Saunders, 1961). Many species, however, ventilate (irrigate) the gills by holding the mouth open while swimming. By analogy with the ramjet engine, this may be termed "ram ventilation." The theory of ramjet mechanics is particularly well understood and may be appropriately applied to analysis of this type of gill ventilation. Through hydrodynamic analysis we have estimated the energy expended in forcing water over the gills during ram ventilation and have related this to the total metabolic expenditure of the fish.

The present analysis is based upon measurements and observations for skipjack tuna (*Katsuwonus pelamis*). Measurements of gill dimensions and geometry were made for this study. The "best available" metabolic data are preliminary, however, and may require correction when more complete measurements are made.

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LIST OF ABBREVIATIONS

A = Flow cross-sectional area.
b = Total span of pectoral fins.
C_o = Initial oxygen concentration in the sea water.
C_f = Friction coefficient.
D_f = Friction drag.
D_w = Drag due to lifting force.
H = Total pressure (equation 3).
L = Fork length or general length along a surface.
l = Average length of the lamellae.
$\dot{O}_2$ = Rate of oxygen consumption.
P = Power (general).
P_g = Power required to overcome gill resistance.
P_p = Power required to overcome total swimming resistance.
p = Hydrostatic pressure.
R = Resistance to forward motion.
Re = Reynolds number, $\rho VL/\mu$.
U = Utilization, fraction of oxygen recovered from sea water.
V = Velocity.
$\dot{V}_g$ = Quantity of water perfusing gills.
W_a = Fish weight in air.
W_w = Fish weight in sea water.
w = Total (summed) width of flow passages.
Δp = Change in hydrostatic pressure across the gills.
ϵ = Efficiency factor for lifting wings or fins.
μ = Vicosity of sea water.
η_t = Efficiency of conversion of chemical energy to mechanical in fish muscles.
η_p = Propulsive efficiency of caudal fin.
ρ = Density of sea water.

Subscripts:
∞ Denoting conditions far ahead of the fish.
x Denoting conditions at the gill exit.

SOURCES OF DATA

The gill from a skipjack tuna was selected from those measured by Muir and Hughes (1969) and examined in further detail. The fork length of this specimen was 44.1 cm and the live weight would have been 1.67 kg (conversion data of Nakamura and Uchiyama, 1966). Total surface area of the secondary lamellae was 30,740 cm^2, total cross-sectional flow area of the channels between secondary lamellae was 250 cm^2, average length of the water pathway through the channels was 0.12 cm, and average thickness of the channels was 0.002 cm.

Basal swimming speed of a skipjack tuna of this size is 66 cm/sec (Magnuson, personal communication). Measurements of the mouth gape of fish of this size swimming at about this speed were made from photographs provided by the Bureau of Commercial Fisheries, Honolulu, Hawaii. Applying these measurements to frozen specimens obtained from the Maryland Tuna Corporation, we estimated that the cross-sectional area of the mouth would be about 1 cm^2 at basal swimming speed.

From earlier measurements of oxygen consumption of skipjack tuna made in the tuna tank facility of the Bureau of Commercial Fisheries Laboratory, Hawaii (B. S. Muir and A. J. Niimi, unpublished data), we estimated that the oxygen consumption ($\dot{O}_2$) of this fish would be 490 ml/hr (0.136 ml/sec) at basal swimming speed. This value is not precise, however, because the measurements were made on larger fish being fed a small daily ration. The oxygen content of the water (C_o) in the tanks during the measurements was 3.5 ml/liter (3.5×10^{-3} ml/ml).

The quantity of water passed over the gill ($\dot{V}_g$) may be determined from knowledge of the fraction of oxygen removed from the water (U = utilization) and the relation

$$\dot{V}_g = \frac{\dot{O}_2}{UC_o} . \tag{1}$$

The water velocity in the mouth must be less than the swimming speed (discussed below), so with a 1-cm^2 mouth area and 66 cm/sec swimming speed, the maximum value for $\dot{V}_g$ is 66 ml/sec. Thus

$$U = \frac{.136}{66 \times 3.5 \times 10^{-3}} = 0.59$$

is a minimum value for the utilization if the other values are correct. Measured values for U range from less than 0.2 to 0.8 (Van Dam, MS, 1938; Hazelhoff, 1938; Saunders, 1962; Hughes and Shelton, 1962; Holeton and Randall, 1967). There may be species variation but the measured values are greatly affected by technique and the metabolic state of the experimental fish. We have assumed 0.7 for the present calculations because it is consistent with our other values and considerations. An error in this assumption does not affect our general conclusions and we show the magnitude of the difference it makes in our final estimates. For U = 0.7, $\dot{V}_g$ = 56 ml/sec. These data are summarized in Table 1.

CALCULATIONS AND RESULTS

Our first task is to define, hydrodynamically, the ram ventilation process so that we can estimate the magnitude of the gill resistance. Conceptually, a "ram" pressure is available from the forward motion of the fish, and we calculate the resistance in terms of loss of this pressure as the water passes through the gill channels. To this pressure loss must be added losses incurred in the mouth and opercular cavities, and the total is expressed as "internal" drag. The energy required to overcome the total internal and external drag is computed and compared with total metabolism as determined from oxygen consumption.

TABLE 1. Measurements, estimates, and assumptions used in the analysis of ram ventilation in skipjack tuna. Sources and derivations are discussed in the text.

	Symbol used in text	Value	Source
1. Fork length	L	44.1 cm	Measured
2. Weight	W_a	1.67 kg	Conversion (Nakamura and Uchiyama, 1966)
3. Frontal cross-sectional area of mouth	A_1	1 cm²	Estimated from frozen specimens and photographs of gape
4. Basal swimming speed	V_∞	66 cm/sec	J. J. Magnuson (personal communication)
5. O_2 consumption at V_∞	$\dot{O}_2$	490 ml/hr 0.136 ml/sec	Estimated from unpublished measurements
6. O_2 content of water	C_o	3.5×10^{-3} ml/ml	Measured in tuna tanks
7. Fraction of O_2 removed from water	U	0.7	Assumed, based on data for other fish
8. Quantity of water passed over gill	$\dot{V}_g$	56 ml/sec	Calculated from 5, 6, and 7
9. Total respiratory surface area	A_g	30,740 cm²	Measured (Muir and Hughes)
10. Total cross section	A_2	250 cm²	Measured
11. Avg length of water path over secondary lamellae	l	0.12 cm	Measured
12. Avg thickness of interlamellar water channel	d	0.002 cm	Measured

ANALYSIS OF GILL RESISTANCE

Consider the situation in which the fish is the reference point and the water is flowing by. Fluid motion is in a steady state so, at any point relative to the fish, speed and motion are not varying with time. Because of the swimming oscillations the mouth is not truly stationary, but these motions should not play a significant role in the ram ventilation process.

The stream tube cross-sectional area increases as the flow passes from the mouth to the level of the secondary lamellae and the consequent decrease in water velocity is determined, using the law of conservation of mass, by

$$\rho\dot{V}_g = \rho AV = \text{constant} \qquad (2)$$

where ρ is the fluid density; $\dot{V}_g$ is the volume flow rate; A is the area of the stream tube; and V is the flow speed.

As shown in the diagram of Fig. 1, there is an increase in the area and a slowing of the stream velocity just ahead of the mouth. This is a result of restricting the opercular slits. Inside the mouth the stream turns and slows further as it passes into the increased area of the slots adjacent to the gill arches. Finally the stream turns 90° and enters the spaces between the secon-

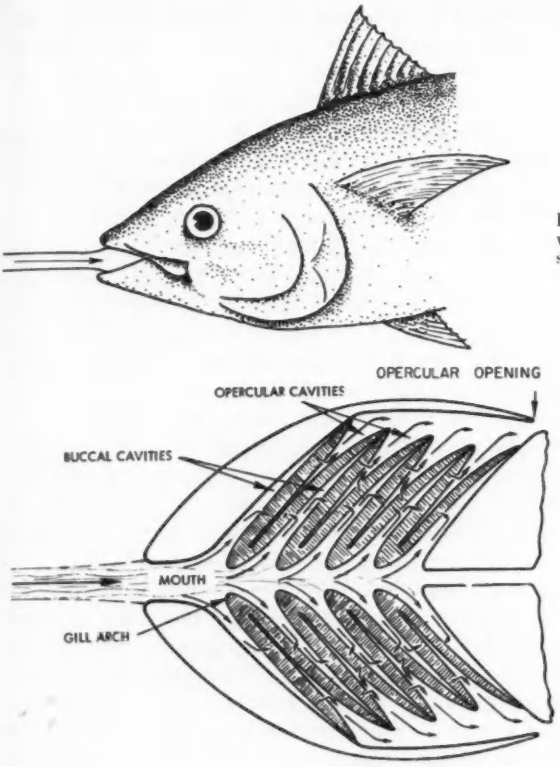

FIG. 1. Respiratory water pathway in skipjack tuna. Upper, sideview; lower, frontal or horizontal section.

dary lamellae. At this level the stream tube is about 250 cm^2 compared with 1 cm^2 at the mouth. From equation 2, the velocity between the secondary lamellae is 0.224 cm/sec, or less than 0.5% of the original. In leaving the gills, the process is reversed and the stream speeds up, but because of energy loss the final velocity of the exiting water is less than the original velocity in front of the fish. The momentum loss so encountered can be directly related to gill resistance.

The analysis of resistance in the gill system requires the use of Newton's law of momentum, the conservation of mass (equation 2), and Bernoulli's equation for the pressure–velocity relation in liquids. The latter equation gives

$$H = \tfrac{1}{2}\rho V^2 + p \qquad (3)$$

where H, the head, is that pressure measured by an open tube faced into the stream of velocity V and pressure p. Note that p is the hydrostatic pressure in the absence of the tube probe. p_∞ is defined as the hydrostatic pressure

far ahead of the fish and is proportional to the swimming depth. For a skipjack tuna swimming at 66 cm/sec at a depth of 10 m, p_∞ is 980,000 dynes/cm^2 and H is 982,232. The maximum "ram" pressure rise is thus 2232 dynes/cm^2, and is more than adequate to provide the necessary flow through the gills.

Fluid friction encountered in passage through the gills results in a sensible reduction in the head pressure, H, from its initial value in the stream. Eventually, far downstream, the fluid returns to ambient pressure, p_∞, but the lowered head results in a lowered velocity relative to V_∞ (equation 3).

Calculation of the net resistance is complicated, however, by several external factors. Because of the shape and thickness of the fish, external water at the region of the gill exits flows slightly faster than V_∞ and results in a local pressure reduction (equation 3). As a consequence the velocity of exiting water is increased over that that would be expected had the pressure been unchanged. A second factor is the passage of the lower velocity respiratory water along the surface of the fish as it mixes with the higher velocity external stream. Contact of the lower velocity water along the body produces a slightly smaller frictional drag than would the higher velocity water. In addition, mixing of respired and external water acts in the manner of a jet pump to reduce energy losses associated with the return of the respired water to ambient pressure.

Finally, there may be favorable interaction of the propelling caudal fin and the lower momentum water that has contacted the body or come from the gills. The caudal fin, or ship propeller, must provide thrust equal to the momentum deficit in the stream. If the propulsor is located in the wake, it directly pumps the low energy water and the final velocity is uniform as shown in the upper panel of Fig. 2. If the propulsor is outside the wake the final flow contains two velocity streams, one having a momentum decrement and the other an equal but opposite momentum increment (lower panel of Fig. 2). Energy involves the square of velocity, however, so a net kinetic

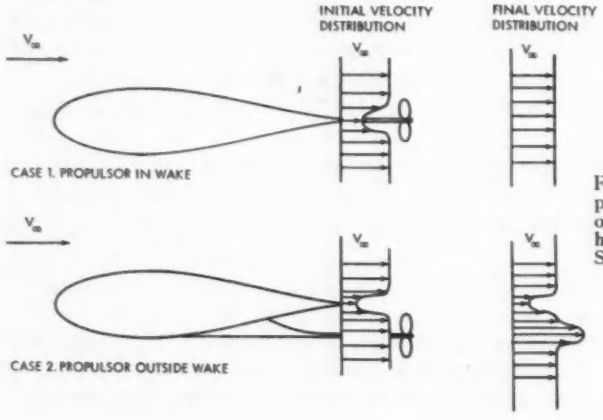

FIG. 2. Interaction between propulsor and the wake of low velocity water behind the propelled body. See text for explanation.

energy increase appears downstream. The caudal fin is well suited geometrically for the first method, which would minimize the energy required for propulsion.

These effects are not included in our analysis because gill resistance is sufficiently small, compared with total resistance, that further refinement in the calculations is not worthwhile. The simplifying assumption that gill water flow and external flow do not mix, allows calculation of an upper limit to gill resistance. Under this condition, internal resistance is a simple function of internal head loss and the result should be adequate to evaluate the magnitude of the energy required for gill ventilation.

Assume the external pressure at the opercular slit is the ambient pressure ($p_x = p_\infty$). Internal resistance is the momentum change, so

$$R = \rho \dot{V}_g (V_\infty - V_x) \tag{4}$$

where V_∞ and V_x are the flow speeds far ahead of the fish and at the exit respectively. Equation 3 may be rewritten

$$V_x = \sqrt{\frac{2(H_x - p_x)}{\rho}}. \tag{5}$$

Defining ΔH as the net loss in head during passage through the gill systems, that is $\Delta H = (H_\infty - H_x)$, and substituting this expression in equation 5, we obtain

$$V_x = \sqrt{\frac{2(H_\infty - p_x)}{\rho} - \frac{2\Delta H}{\rho}}. \tag{6}$$

Making use of equation 3 and the assumption that $p_x = p_\infty$, equation 4 can be written

$$R = \rho \dot{V}_g V_\infty \left[1 - \sqrt{1 - \frac{2\Delta H}{\rho V_\infty^2}}\right] \underset{\Delta H \to 0}{\simeq} \frac{\dot{V}_g \Delta H}{V_\infty}. \tag{7}$$

Equation 7 shows that the gill resistance at a given speed is directly proportional to the quantity of water flowing over the gills, $\dot{V}_g$, and almost proportional to the internal loss, ΔH.

Head Loss in the Gills

The head loss, ΔH, which appears in the resistance equation 7, must be calculated from the estimated flow rate, surface area, and lamellar geometry.

The cross-sectional area at the level of the secondary lamellae is so great (250 cm^2) that the flow rate is very small. From equation 2

$$V = \frac{\dot{V}_g}{A} = \frac{56}{250} = 0.224 \text{ cm/sec.} \quad (8)$$

At this velocity, the kinetic energy term of equation 3 (the difference between the head, H, and the static pressure, p),

$$\tfrac{1}{2}\rho V^2 = \frac{(0.224)^2}{2} = 0.025 \text{ dyne/cm}^2,$$

is negligible, so the static pressure loss across the secondary lamellae will also be the head loss.

The pressure loss occurring as the flow passes through the lamellar channels can be estimated by use of the Poiseuille relation (Poiseuille, 1849)

$$\Delta p = \frac{12\mu\ Vl}{d^2} \quad (9)$$

where μ is the viscosity of the water; V is the mean speed; l is the channel length; and d is the average thickness of the channel. Thus the head loss is

$$\Delta H = \Delta p = \frac{12(.01)\ (.224)\ (.12)}{(.002)^2} = 800 \text{ dynes/cm}^2$$

where the viscosity μ is taken as .01 poise. The value of 800 dynes/cm^2 corresponds to about 0.8 cm of water, a value not in disagreement with pressure drops across the gills of "pumping" fish (Hughes and Shelton, 1958; Saunders, 1961).

The dimensions used to calculate the pressure loss are subject to error beyond those of simple measurement. The diagram in Fig. 3 represents a cross section through two filaments and shows the surface of two secondary lamellae. The secondary lamellae are fused for part of their length (Muir and Kendall, 1968) and the flow of blood is generally obliquely across the lamellae (Muir, 1970). The secondary lamellae are curved out of plane giving a variable thickness to the water channel that could direct the water flow so that it also passes obliquely but counter-current across the surface of the lamellae. Assuming this to be the case, we took l = 0.12 cm for the average water pathway depicted in Fig. 3. However, such a phenomenon implies an increased pressure drop. For example, a simple bending of the lamellae to an angle of 30° would narrow the channel beyond the bend by 13% and produce a pressure loss increment of about 26%. We have not attempted to take the effect of curvature into account in calculating the pressure drop.

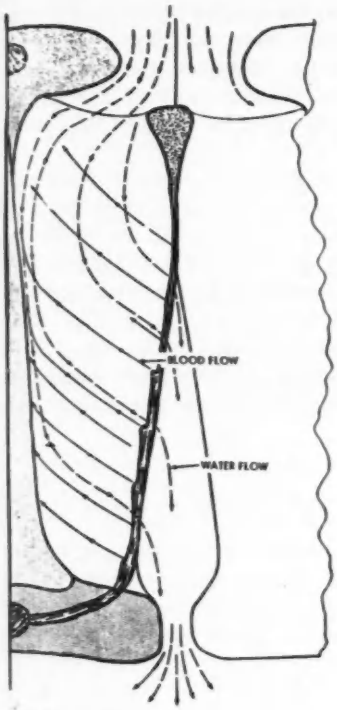

FIG. 3. Diagramatic cross section through two adjacent gill filaments showing the blood pathway across a secondary lamella and the assumed water pathway. Note the fusion of secondary lamellae.

In addition, the thickness of the water channel may be altered by changes in the thickness of the secondary lamellae. The pillar cells (which form the "skeleton" of the secondary lamellae) may undergo expansion and contraction, which could change the thickness of the blood spaces (Plehn, 1901; Hughes and Grimstone, 1965; Newstead, 1967; Munshi and Singh, 1968). Such change would necessarily require an opposite change in the thickness of the water channel.

HEAD LOSS IN THE MOUTH AND OPERCULAR CAVITIES

The pressure drop of 800 dynes/cm^2 incurred in the lamellar channels is only part of the total loss. To this we must add the pressure loss in the mouth and opercular cavities.

At the entrance to the mouth, the area of the stream tube increases to 1 cm^2 and the velocity decreases to 56 cm/sec. The reduction from 66 to 56 cm/sec results in an external pressure rise of about 625 dynes/cm^2 (equation 3), there being no head losses in the region ahead of the mouth. Head losses due to viscosity arise as the water scrubs the walls of the mouth cavity and surfaces of the gill arches. The presence of the gill lamellae and their attendant

large pressure drop tends to stabilize the flow ahead (Schubauer and Spangenburg, 1948), but some eddy formation results from the slowing down in the mouth and produces head losses. Engineering experience with such flows suggests that losses amounting to 15% of the velocity head at the mouth should be anticipated. The head loss is thus estimated to be (15% of 2232–625) 240 dynes/cm^2.

Losses in flow exits are generally small and result primarily from viscous shear on the walls. For round exits, less than a few percent of the exit kinetic energy are encountered. We have taken 5% because the opercular exit is a slit and has a greater surface area (5% of 2232 – (800 + 240) = 60). The total pressure head for the exit water is therefore 1132 and the head loss for the entire system is 1100 dynes/cm^2.

The head loss profile for the case under study is depicted in Fig. 4 as a pressure–velocity diagram. Starting at the ambient pressure and 66-cm/sec point, the flow slows and pressure increases progressively to the gills where

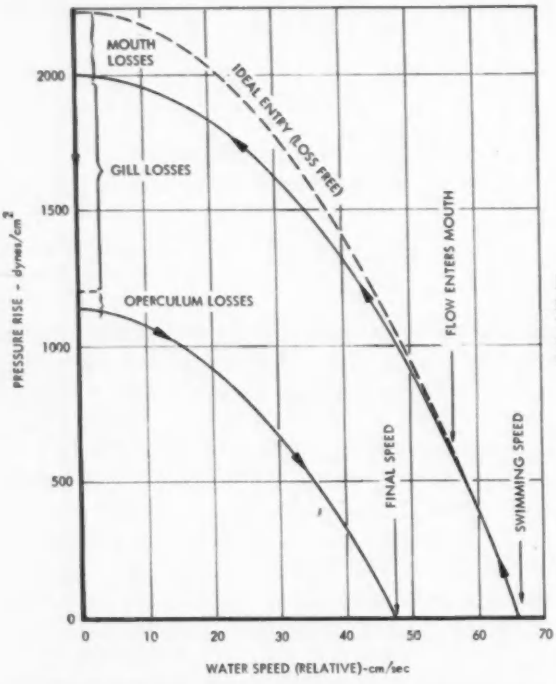

FIG. 4. Pressure–velocity diagram depicting changes in water velocity and pressure head as respiratory water flows through the mouth, gills, and opercular cavity.

velocity, to the scale plotted, is essentially zero. Had there been no mouth losses the pressure would have risen as shown by the dotted line labelled "I deal entry." In the gill the greatest loss is incurred and finally after a small head loss in the operculum the pressure returns to ambient with a velocity of 47 cm/sec at the exit slit.

Substitution of the total pressure head loss computed in the resistance equation 7 and use of the values in Table 1, obtains for us the overall gill resistance

$$R = 1.025 \times 56 \times 66 \left[1 - \sqrt{1 - \frac{2 \times 1100}{1.025(66)^2}} \right] = 1090 \text{ dynes.}$$

Estimation of Total Resistance

The resistance of the negatively buoyant tuna can be broken into the following categories: (1) body friction; (2) pectoral and caudal fin friction; (3) drag due to lift; (4) body and fin-form drag; (5) gill resistance.

Drag components were estimated using accepted analogous principles (Hoerner, 1958). The onset of turbulent eddying flow at surfaces is generally determined by the Reynolds number, which is defined

$$Re = \frac{\rho VL}{\mu}$$

where ρ, μ, and L are water density, water viscosity, and a characteristic length of the surface, respectively. For values under 300,000 that are pertinent to our problem it is generally found that laminar flow prevails.

The friction drags were computed by the formula:

$$D_f = \frac{C_f \rho V^2 A}{2}$$

where D is the drag, ρ is the water density, V the swimming speed, A the total wetted area of the part, and C_f a friction factor depending on the Reynolds number for the part. The characteristic length of the fins was taken as the average length in the direction of motion. The expression for friction coefficient is

$$C_f = 1.34/\sqrt{\text{Re.}}$$

The values used for the present estimate and the resulting component drags are given in Table 2.

The form drag is a basic resistance arising from pressure forces on the body and fins. It is associated with the viscous resistance and includes the effect of increased friction due to locally higher flow speeds as well as energy losses due to mixing in the wake.

The form drag for the body can be inferred from test data at the correct Reynolds number collected by Hoerner (1965). For the ratio of length to mean diameter of the skipjack tuna of 4.5 the value is 25% of the basic body friction drag or 1250 dynes. The form drag of the fins carrying lift can be assigned

TABLE 2. Estimation of friction drags for body and fins.[a] Methods of calculation discussed in text.

Part	L (*cm*)	Re	C_f	A (cm^2)	D_f (*dynes*)
Body (fork length)	44	290,000	0.0025	890	5,000
Caudal fin	2.2	14,600	0.011	62	1,520
Pectoral fin	1.5	9,900	0.014	35	1,100
Dorsal and ventral (mean)	1.3	8,600	0.015	15	500

[a]Retractable and pelvic fins not considered.

a value of 80% of the friction drag on the basis of data for cambered flat plates carrying lift at a Reynolds number of 10,000. The pectoral fins maintain a substantial inclination or "angle of attack" to the stream and therefore the form drag is much greater than would be expected for a noninclined fin. Because the caudal fin carries an average inclination somewhat less than the pectorals and the dorsal and pelvic fins none, an average estimate for the total fin-form drag has been taken as 50% of the friction drag or 1560 dynes.

The drag due to lift arises because the pectoral fins and body are supporting the major portion of the unbalanced weight and buoyancy forces and this production of lift requires (on basic principles) vortices to be created at the tips and rear edges of the pectorals. The energy associated with these vortices appears as an additional drag force on the fin. A general formula originally given independently by Lanchester and Prandtl can be found in books on aeronautics as:

$$D_W = \frac{2W^2}{\pi \epsilon \rho V^2 b^2}$$

in which W is the lift force generated by the wing, b is the total breadth or span of the wing, and ϵ is an efficiency factor usually known to be between .8 and 0.9. The lift, W_w, required for our tuna can be obtained from the relation given by Magnuson (1970):

$$W_w = W_a \left(1 - \frac{\text{specific gravity of sea water}}{\text{specific gravity of the fish}}\right).$$

Specific gravities are 1.025 for sea water and 1.09 for the fish and the fish weight in air was 1.67 kg, so the lift required, W_w, is 97,600 dynes. With the pectoral fins fully extended, the distance from tip to tip (b) was 24 cm. ϵ is taken as .85 on the assumption that the lift is distributed in an efficient way. With these values, D_w = 2800 dynes.

All fundamental components of the resistance are now at hand and are summarized in Table 3.

TABLE 3. Summary of the component drags calculated for a 44-cm skipjack tuna swimming at a speed of 66 cm/sec.

Component	Drag (*dynes*)
Body friction drag	5,000
Fin friction drag	3,120
Body-form drag	1,250
Fin-form drag	1,560
Drag due to lift	2,800
Gill drag	1,090
Total swimming resistance	14,820

The gill drag, 1090 dynes, represents only about 7% of the total swimming resistance and as computed would vary as the square of the quantity of respiratory water $\dot{V}_g$ (see Discussion).

ENERGY CONSIDERATION

The energy available from the oxygen recovered can be estimated by taking the caloric value of oxygen as 5 cal per ml. For an oxygen consumption of .136 ml/sec (Table 1) we obtain a net energy equivalent of

$$P = .136 \text{ ml/sec} \times 5 \text{ g cal/ml} = .68 \text{ g cal/sec},$$

which is the total power available for all functions.

Using the resistance values from the previous section, we may calculate the energy required for both respiration and swimming. The portion of the total power used for overcoming resistance is affected by two efficiency factors, the conversion of chemical energy to mechanical work in the muscles, and the efficiency of converting mechanical work in the swimming muscles to propelling power. Reasonable values for these factors, η_t and η_p, may be taken as .25 (Lehninger, 1965) and .8, and we may write for the propulsion power required

$$P_p = \frac{D \times V}{\eta_t \times \eta_p} = \frac{15000 \times 66}{.25 \times .8} = 5 \times 10^6 \text{ ergs/sec} = .12 \text{ g cal/sec}.$$

Thus, of the 0.68 g cal/sec available energy, approximately 0.12 g cal/sec (about 17%) are used for swimming.

Finally, we return to the cost of respiration and in terms of g cal/sec the 1090 dynes resistance becomes

$$P_g = \frac{1090 \times 66}{.25 \times .8} = 3.6 \times 10^5 \text{ ergs/sec} = .009 \text{ g cal/sec},$$

a value that is slightly over 1% of the total metabolic energy available from the oxygen extracted.

DISCUSSION

Ram ventilation is not restricted to the tunas so the above analysis has wider application. The tunas (scombroids generally), however, have largely lost the ability to pump sufficient water over the gills (Hall, 1930; Magnuson, 1963). Being negatively buoyant, they must swim continuously for hydrostatic equilibrium (Magnuson, 1970) and the reliance on ram ventilation may be a consequence of this. It is observed in other species, e.g., salmon (Smith et al., 1967; Stevens and Randall, 1967), when the rate of forward motion is adequate. It is also used by the remora (*Remora remora*) when attached to the wall of a water tunnel (Muir and Buckley, 1967) and presumably when riding on a host fish. The advantage to the remora is obvious, for the host fish provides the driving power. For free-swimming fish the advantage of ram ventilation is likely the minimization of further expenditure in the form of external energy loss resulting from pumping during forward motion.

Among the scombroids there is variation in the mouth position. In mackerel the mouth is almost continuously slightly agape and the gape increases in response to increased oxygen demand after feeding. If the oxygen content of the water is lowered, the gape increases further and continuously varies (personal observation). In other species, alternating open and closed seems normal and the response to increased oxygen demand is a greater "mouth-open" time (Magnuson and Prescott, 1966; J. J. Magnuson, personal communication). Skipjack tuna tends to hold a constant gape at a given oxygen consumption (J. J. Magnuson, personal communication) so our assumption of a steady state is reasonable.

Our basic model represents as close an approximation to reality as is likely possible. Errors associated with measurements and hydrodynamic assumptions appear to be relatively small and further refinement would not greatly alter the results.

The greatest errors result from the oxygen consumption and utilization estimates and these, fortunately, can be checked and corrected when improved data are available. As was pointed out (equation 1) the estimate of the quantity of water passed over the gill ($\dot{V}_g$) depends on the oxygen consumption and the fraction of oxygen removed from the water (U). Even if there were no velocity drop at the mouth, the value of U could not be lower than 0.59 with the given oxygen consumption. Recent work by D. J. Randall and students (personal communication) suggests that the value for rainbow trout may be 0.45 or less. Tuna, of course, could be more efficient.

It is possible, however, that the value of 0.136 ml/sec for oxygen consumption ($\dot{O}_2$) is too high. A lower value would allow a smaller U for the same $\dot{V}_g$. Only $\dot{V}_g$ was used for the calculations of gill resistance so the estimated cost of respiration remains at 0.009 g cal/sec. If the oxygen consumption were

only half of the value we used, this cost of respiration would still be only about 3% of the total metabolism (0.009/0.34). Similarly the cost of swimming estimate would not change, but it would become a larger percentage of the total metabolism.

In comparison with our present estimate of total metabolism (0.68 g cal/sec), the standard and active (maximum) oxygen consumption measurements for a 1.7-kg salmon starved for 36 hr (Brett, 1965a) convert to about 0.069 and 1.18 g cal/sec. The cost of swimming 66 cm/sec for a salmon of about this weight is approximately 0.16 g cal/sec (Brett, 1965b), which is comparable to our estimate of 0.12 for the tuna.

Oxygen consumption can be increased by increasing the mouth gape and $\dot{V}_g$, but this results in a greater gill resistance. For $\dot{V}_g$ = 56 cm/sec the total pressure drop was 1100 dynes/cm^2 (800 in the gills and 300 in the mouth and opercular cavity). Doubling $\dot{V}_g$ would approximately double the pressure drop and bring it up to nearly the ram pressure (2232 dynes/cm^2), which surely limits $\dot{V}_g$ at the given swimming speed. If the pressure drop increases from 1100 to 2000 dynes/cm^2, then the total gill resistance increases to about 5000 dynes. The gill resistance is now about 27% of the total swimming resistance rather than just 7% (Table 3) and the cost of swimming increases by 26%.

We have substantiated this experimentally with mackerel (to be reported later) by reducing the oxygen content of the water. While maintaining approximately the same swimming speed, the mackerel progressively increase the mouth gape as the oxygen level is reduced. The tail-beat frequency and the oxygen consumption increase, reflecting the additional work required to overcome the extra gill resistance.

Beyond the limit of the given ram pressure head, the fish may increase $\dot{V}_g$ by increasing swimming speed — thus developing a higher ram pressure. But the cost of swimming quickly rises and although more oxygen is made available, an increasing portion of it is required for the acquisition.

ACKNOWLEDGMENTS

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NOTES

Effect of Solitude on Time Between Fourth and Fifth Larval Molts in the American Lobster (*Homarus americanus*)

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Larvae held individually in containers spent an average of 12 days in the fourth stage. When held two larvae per container, one molted after an average of 12 days, the second after an average of 16 days. Duration of the fourth stage was not affected by gravel, shelter or bare bottom conditions, or illumination varying from <1 lux to 310 lux.

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IN A PREVIOUS note (Cobb, 1968), I showed that larvae of the American lobster held in groups of five spent less time in the fourth stage when held over gravel bottoms than when held over bare, sand, or mud bottoms. In this study I attempted to determine what factor of the gravel bottom conditions had affected the duration of the fourth stage. Larvae were held singly or in pairs in containers supplied with gravel, shelter, or nothing on the bottom and were subjected to either very dim or somewhat brighter light.

Materials and methods — The experiment was run July 2–19, 1968, and repeated in part July 2–17, 1969, with some modification.

Larvae were held in plastic freezer containers 8 cm on a side and 12 cm deep for single-animal treatments and 11 × 11 × 16 cm for pairs. The three bottom conditions were rounded beach gravel 2–4 cm in diameter; opaque polyvinylchloride hemicylinders 3.5 cm long and 2.0 cm wide for shelter, one per larva in the shelter treatments; and bare bottom. In 1968 the two conditions of illumination inside the containers were 10–37 lux at midday depending on the position of the container on the table; and less than 1 lux at midday, a small amount of light being allowed in to cue the larvae to the photoperiod. In 1969 the natural light conditions were augmented with two 20-w cool-white fluorescent lamps set to turn on ½ hr after sunrise and off ½ hr before sunset. Illumination varied from 200 to 310 lux in the bright light treatments and was <1 lux in the dim treatments. Topless, unpainted containers were used for the bright treatments; they were painted flat black and supplied with tops for the dim treatments. Illumination was measured with a Tsurumi-Seiki submarine illuminance meter.

Approximately 0.4 liter of sea water was placed in the single-larva containers and 0.8 liter in the two-larvae containers. The water was not changed during the experiment. The larvae were fed brine shrimp nauplii daily, the two-larvae containers receiving twice as much as the

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single-larva containers. The containers were set in a running seawater bath to maintain uniform temperature. The average temperature was 19.0 C (17.6–20.8 C) in 1968 and 19.9 C (18.0–21.7 C) in 1969.

Lobster larvae were obtained from the Massachusetts Lobster Hatchery and randomly assigned to the experimental containers. In 1968, fourth stage larvae stated to have begun molting the previous day were obtained. In 1969, third stage larvae about to molt were taken and held in 20-gal aquaria, and only the larvae molting to the fourth stage during a 26-hr period were used in the experiment.

The 1969 experiments were performed as a check on the essentially negative results from the effects of light and bottom conditions obtained in 1968, so the pair treatments were omitted.

The number of days from fourth to fifth molt was measured. In 1968, 16 larvae were started in each of the single-larva treatments and 20 (10 pairs) in each of the two-larvae treatments. Fifteen larvae were started in each treatment in 1969. A $3 \times 2 \times 2$ (1968) or 3×2 (1969) factorial design was employed in a randomized complete block and the data were subjected to a multivariate analysis of variance computer program for balanced data (Hemmerle et al., MS, 1969). Mortality during the experiment resulted in unequal numbers of replicates, so values that minimized the experimental error sum of squares were automatically substituted during the analysis for the missing data points. The analyses were performed with a rejection level of $\alpha = 0.05$.

Much of the mortality occurred in the gravel treatments and may have been due to the larva being crushed between stones as I searched for it. In the two-larvae treatments, if one larva died before either had molted the data were discarded; but if one died after either had molted, the day of molt was included in the data.

Results — The mean numbers of days ± 95% confidence limits (N = 12–19) spent in the fourth larval stage under the various conditions in 1968 and 1969 were

	Lux	Shelter	Gravel	Bare
One larva	10–37	12.13±0.82	11.69±0.87	11.94±0.71
(1968)	<1	11.13±0.49	11.62±0.72	11.81±0.59
Two larvae	10–37	14.27±1.65	13.00±1.88	14.42±1.65
(1968)	<1	13.44±1.21	13.79±1.87	14.24±1.27
One larva	200–310	12.47±1.24	12.00±1.20	12.60±1.16
(1969)	<1	11.93±1.27	11.42±1.20	11.80±1.01

In 1968 the larvae in the two-larvae treatments spent a significantly longer time in the fourth stage than those in the single-larva treatments. The numbers of days in the fourth stage averaged as follows: single larvae, 11.77; first of two, 11.82; and second of two, 16.08 (Fig. 1). Neither in 1968 nor in 1969 was there a significant effect of bottom or illumination conditions.

The confirmation by the 1969 experiments of the lack of effect of light or substratum on time between molts clearly leaves the interaction between larvae as the only factor affecting intermolt time in these experiments.

Discussion — Solitude apparently is an important factor in the regulation of molting in *Homarus americanus* larvae as has been shown for the adult lobster (Stewart and Squires, 1968) and *Gecarcinus lateralis* (Bliss and Boyer, 1964). The interaction between larvae causing a delay of molt apparently was not affected by light, shelter, or gravel. The shape of the second-larva

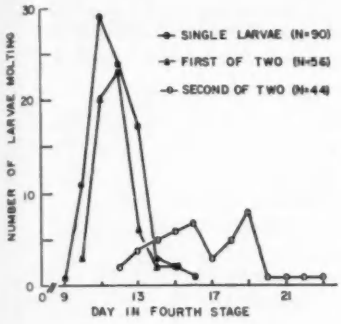

FIG. 1. Numbers of larvae molting from fourth to fifth stage each day, 1968 experiment.

molting curve (Fig. 1) is revealing; there is a dip at day 17, which could be interpreted as the time of effect of an inhibitory agent. The similar shape of the molting-frequency curves of the single-larva treatment and the first larva to molt in the two-larvae treatment suggests that the interaction between larvae may take effect at or after the time of molt of the first larva. The nature of the interaction has not been determined, but a dominance relation with competition for food and space, or a chemical released near the time of molt, could be hypothesized. Possibly, in the experiments reported previously (Cobb, 1968), the gravel and a greater bottom area per larva provided enough isolation so molting occurred earlier in the gravel treatments.

The results presented here appear to contradict Hadley's (1906) and Templeman's (1936) views that bright light inhibits the growth of larval lobsters. However, the illumination used was probably lower than Hadley's or Templeman's. Thus there may be an illumination threshold below which light has no effect on growth and molting of larval lobsters.

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Seasonal Depth Distributions of Landlocked Atlantic Salmon, Brook Trout, Landlocked Alewives, and American Smelt in a Small Lake

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LACKEY, R. T. 1970. Seasonal depth distributions of landlocked Atlantic salmon, brook trout, landlocked alewives, and American smelt in a small lake. J. Fish. Res. Bd. Canada 27: 1656–1661.

Seasonal depth distributions of landlocked Atlantic salmon (*Salmo salar*), brook trout (*Salvelinus fontinalis*), landlocked alewives (*Alosa pseudoharengus*), and American smelt (*Osmerus mordax*) were determined monthly in Echo Lake, Maine, using vertical and horizontal gillnets.

Salmon were wide-ranging fish, but generally not captured in very shallow or very deep water. Brook trout were primarily an inshore species, not often captured in water deeper than 25 ft, and nearly always found close to the lake bottom. The majority of captured alewives were taken from shallow to middepths (0–30 ft) in summer and fall and in deep water during winter and spring. Smelts were widely distributed, but the majority were captured in water deeper than 30 ft every month.

No clear temperature or dissolved oxygen preference could be shown for any of the four species.

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FEW SEASONAL DEPTH DISTRIBUTIONS of fish species have been intensively studied. One technique to determine depth distribution is the use of vertical gillnets. Tibbles (MS, 1956) used this method with success to study the distribution of yellow perch (*Perca flavescens*) in Lake Mendota, Wisconsin. Horak and Tanner (1964) reported the results of vertical gillnetting in a Colorado reservoir to determine the summer distributions of rainbow trout (*Salmo gairdneri*), kokanee (*Oncorhynchus nerka*), white sucker (*Catostomus commersoni*), and yellow perch (*P. flavescens*). The distributions of white catfish (*Ictalurus catus*) and rainbow trout were determined with vertical gillnets by von Geldern (MS, 1964) for a California lake.

The purpose of this study was to determine seasonal depth distributions of four fish species inhabiting Echo Lake, Maine, from June 1967 to May 1968, using both vertical and horizontal gillnetting. Water temperature and dissolved oxygen were measured to evaluate their effect on fish distribution.

Study area — Echo Lake, located on Mount Desert Island, Maine, is partially in Acadia National Park. Surface area is approximately 237 acres (96 ha) and the maximum depth is 63 ft (19.2 m). The lake is oligotrophic and of glacial origin (Fig. 1).

Landlocked salmon were introduced in 1965 as age 0+ fall fingerlings. Brook trout were stocked periodically as fingerlings (age 0+). Adult land-

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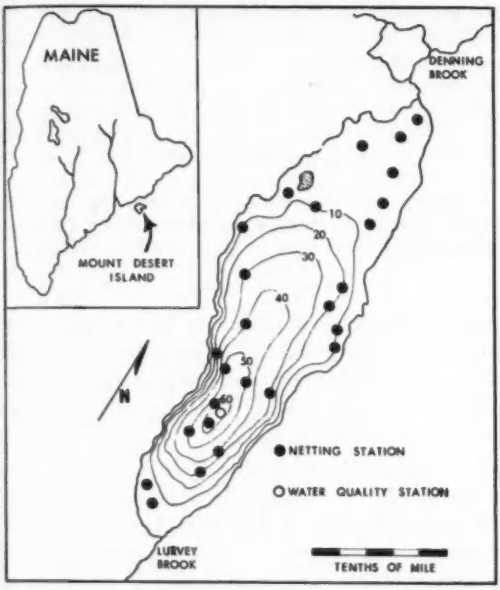

FIG. 1. Echo Lake, Mount Desert Island, Maine, showing depth contours in feet and sampling stations.

locked alewives from Cayuga Lake, New York, were introduced in June 1966, and successfully spawned that summer and each summer since. A smelt population was maintained by natural reproduction.

Materials and methods — Small-meshed vertical gillnets (02 and 03 filament nylon with ¼- and ½-inch bar mesh, respectively) were designed and built (Lackey, 1968) to determine the depth distributions of small fish in Echo Lake. Six lengths of nets were used. The numbers of ¼- and ½-inch mesh of each length were as follows:

	Length (*ft*)					
	10	20	30	40	50	60
¼-inch	3	3	1	1	1	1
½-inch	3	3	1	1	1	1

Twenty-six sampling stations (Fig. 1) were selected at depths corresponding to the above six net lengths. The number of stations at each depth was roughly proportional to the lake surface over that depth. Selection of sampling stations was affected by several considerations, including their proximity to swimming and boating areas. Each station was marked by two large rock anchors separated by a distance twice the depth (Lackey, 1968).

During summer months, 14–19 nets were set for 48 consecutive hr each week. Each net was cleared of fish at least every 12 hr and usually more frequently. Captured fish were recorded by species, length, and depth of capture.

During winter sampling, ice was cut with a chain saw to permit placement of gillnets or, if ice was thick, with a gasoline-powered ice auger. Sampling during these months was limited to once or twice per month.

Although a few salmon and trout were captured in vertical gillnets, concurrent sampling with horizontal gillnets was necessary for more accurate estimates of the distributions of these species. Two 500-ft gangs, each composed of five 100-ft sections (8 ft deep) of different mesh sizes (1½-, 2-, 2½-, 3-, and 4½-inch stretch mesh), were used to determine depth distributions. To estimate depth of capture with these nets, it was necessary to make sets with a fathometer or in areas of known depth. By setting both gangs perpendicular to shore, it was possible to sample all depths simultaneously. Usually both nets were moved to another location during the second 24 hr of the 48-hr sampling period.

Water temperature profiles were taken with a custom-made electric resistance thermometer. Dissolved oxygen was determined by the Alsterberg modification of the Winkler method (American Public Health Association, 1965). Determinations were made at 10-ft intervals during each sampling period.

Results — Distribution data show landlocked salmon to be a wide-ranging fish (Table 1). During most of the year it was observed that salmon were not captured near the lake bottom. The exceptions to this were shallow-water catches in October and deep-water catches in April and May. There is also a distinct increase in depth of capture as summer progresses.

Brook trout in Echo Lake are generally residents of the upper 25 ft of water, but not limited to this range (Table 1). This species was most commonly captured in the lower several feet of gillnet at a given station. It was also observed that captures were highest at stations nearest shore. As with salmon, there is a distinct increase in depth of capture in August.

Alewives were usually captured in nets set in deep water. In summer and fall, alewives occupy the upper depths of the lake, mainly away from shore and bottom (Table 1). With the onset of winter, there is a marked movement to deeper waters, where the species remains until the following summer.

Smelt depth distribution followed no clear pattern (Table 1), except for an increase in depth of capture as summer progressed.

Temperature and dissolved oxygen preferences could not be shown for any of the four species.

Discussion — Landlocked salmon, unlike brook trout, are a wide-ranging fish and favor open water, away from the lake bottom. Feeding habits of salmon (Lackey, 1969) in Echo Lake also indicate a distinctly pelagic existence. Salmon in Echo Lake tended to move to deeper water as summer progressed. Similar movement of salmon has been shown in two Newfoundland ponds (Leggett and Power, 1969).

Brook trout in Echo Lake are generally near the lake bottom when captured, as reported by Flick and Webster (1962) in a New York lake and Chapman et al. (1967) in an Oregon lake. Further, food habits indicate much inshore bottom foraging (Lackey, 1969).

Depth distribution of alewives in Echo Lake follows the general patterns previously described (Galligan, 1962; Gross, 1953, MS, 1959; Reigle, 1969), but differs somewhat in timing. Seasonal differences account for this. In summer and fall, alewives occupy the upper depths of the lake, mainly areas away

TABLE 1. Depth distributions of landlocked Atlantic salmon, brook trout, landlocked alewives, and American smelt based on combined vertical and horizontal gillnetting in Echo Lake, 1967–68.

	1967							1968				
Depth (*ft*)	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Landlocked Atlantic Salmon												
0–5		1	1		22			1				1
5–10	1	2		2	5					1		1
10–15				2	2			1				
15–20			1									
20–25	2		2			2						
25–30	1	1	1	1		4						
30–35	1	4	2									
35–40	1	7	4	1								
40–45		1										1
45–50			1									1
50–55			3	2								3
55–60			1	1							5	9
Brook Trout												
0–5		1			3			6			2	3
5–10		3		1				6	1		1	2
10–15		3		1	1			1				
15–20		4										
20–25			4			3						
25–30				1		2						
30–35			1									
35–40												1
40–45												1
45–50												
50–55			1									
55–60			1									2
Landlocked Alewives												
0–5		2	10			3				3		
5–10	34	97	6							3		
10–15	23	32	13	1		200				4		
15–20	35	15	1		3					3		
20–25	21	5	6		1					5	4	
25–30	10		4				1			5	4	
30–35		2		1	1					22		
35–40		5	1	2				15		21		
40–45								38		69		
45–50					1			38		68		
50–55	1							39		69	1	2
55–60								40		71	57	3
American Smelt												
0–5					2							1
5–10	4	16	3		2							
10–15	7	2			1							
15–20	8	3	2	3	2							
20–25	11	1	1		2							
25–30	16	6	5		2	4						
30–35	35	49	22		7							
35–40	38	69	26	1	4							
40–45	4	3	18	2	8							
45–50	5	10	30	3	5							
50–55	1		9		1						1	
55–60			11								2	1

from shore and bottom. With the onset of winter, there is marked movement to deeper waters, where the species remains until the following summer.

Galligan (1962) studied the distribution of alewives in Cayuga Lake, and reported a pronounced inshore movement in late June and July associated with shallow-water spawning habits. During late summer and fall, Cayuga Lake alewives moved to deeper water. Galligan hypothesized that alewives took up a pelagic distribution in winter. Gross (1953, MS, 1959) reported similar findings in Lake Hopatcong, New Jersey.

Reigle (1969) studied alewife distribution in southern Lake Michigan with a bottom trawl. His results are similar to those reported here even though sampling methods were different. From January to April, alewives were captured in the deeper water of Lake Michigan (35 fath or deeper). In April and May alewives were taken over a wide range of depths. During spawning season (mid-May through June), alewives were found only in shallow water. Trawl catches decreased during July, August, and September, which indicated a vertical scattering of alewives. Trawling later in the year showed alewives in water 0–35 fath. Reigle reported a pronounced movement into deep water in December and January.

None of the four species studied showed a clear preference for any particular temperature, although temperature preference was difficult to evaluate because of an extreme annual range. The only consistent trend that might be associated with temperature was the progressively greater average depth of capture as summer progressed, then a rise in depth of capture in early fall.

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Food of Flathead Sole (*Hippoglossoides elassodon*) in East Sound, Orcas Island, Washington

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MILLER, B. S. 1970. Food of flathead sole (*Hippoglossoides elassodon*) in East Sound, Orcas Island, Washington. J. Fish. Res. Bd. Canada 27: 1661–1665.

Flathead sole (*Hippoglossoides elassodon*) in East Sound fed on both benthic organisms (clams, polychaetes, and pea crabs) and nektonic organisms (mysids, fishes, and shrimps). The smallest sole (40–179 mm total length (TL)) ate primarily mysids, but as the fish increased in size they utilized first shrimps and then fishes (mainly herring) and some clams. All except the largest sole (320–440 mm TL) also continued to feed heavily on mysids. Fish larger than 320 mm had the highest number of empty stomachs during the warmer months of the year whereas those smaller than 320 mm had the highest number empty during the colder months. In mature fish, the number of empty stomachs increased during the peak of spawning.

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THE FOOD HABITS of the flathead sole, *Hippoglossoides elassodon* Jordan and Gilbert, have not previously been described in detail. Suyehiro (1934), Smith (1936), Ellson et al. (1950), Clemens and Wilby (1961), and Skalkin (1963) indicate that this fish feeds on both benthic organisms such as polychaetes, molluscs, and brittle stars, and nektonic organisms such as fishes and shrimps. This report describes the changes in the diet of the flathead sole in East Sound, Orcas Island, Washington, by size and season.

Methods and materials — East Sound is a long and shallow (about 15 fath) embayment characterized by sluggish circulation and a bottom sediment of silty mud, moderately compacted. Fishes and invertebrates found in the study area have previously been indicated (Miller, 1967).

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Collecting was done by several types of trawls varying in mesh size in the codend from ¼- to 1¼-inch. Most of the fish were caught during monthly collections from February 1965 to January 1966, but additional fish in age-groups 0 and I were caught during monthly samples taken in 1966. All collecting was done during daylight hours since preliminary observations indicated the sole were probably visual daytime feeders.

Captured fish were immediately killed by a sharp blow on the head; the abdominal wall was slit; and the fish were placed in 10% neutral formalin. During this operation fish were classed as immature, mature, ripe, or spawned out.

Temperature and depth were obtained with a bathythermograph.

After 2 months or more, the preserved sole were measured for total length and the stomachs removed. The stomach was taken as the digestive tract from immediately posterior to the pylorus to as far anterior as it was possible to cut with a pair of curved surgical scissors. Stomachs were grouped by month of capture and 20-mm size groups, the sexes and the 2 years of capture being combined. For each month and size group, the number of stomachs in which the food item occurred was recorded, and the combined wet weight (blotted to remove excess water) of the food item in the size category was obtained to the nearest 0.1 g. Shells of molluscs were omitted from the weights. Empty stomachs were recorded.

In analyzing the food data, the frequency of occurrence (number of stomachs a food item was found in) and the gravimetric systems were used, expressed as percent occurrence and percent weight. Empty stomachs were not included in either calculation. The March sample was missing for all but the smallest size category.

Food analysis data were plotted against month of capture and against the 20-mm size groups. On the basis of these plots, the sole were divided into four length groups (which reflected the changes in diet) for presentation of the food data.

Results and discussion — The detailed results are to be found in Miller (MS, 1969). The present report gives only the general findings.

A gross description of the food habits of the sole was obtained by lumping lengths and months (Table 1). Of the 1952 stomachs examined, 30.6% were

TABLE 1. Stomach contents of flathead sole, all lengths and all months combined.

Food item	Total no.	% occurrence in stomachs	W(g)	% of total wt
Mysids	1048	77.4	279.1	35.9
Shrimps	129	9.5	119.3	15.3
Clams	69	5.1	45.0	5.8
Fishes	132	9.7	300.3	38.6
Polychaetes	106	7.8	21.8	2.8
Other	123	9.1	12.3	1.6

empty. Nektonic organisms were found in 84% of the stomachs and made up about 91% of the total weight of the contents. Mysids were the most numerous food item and about equalled fishes in weight. Generally, fishes appeared to be second in importance to mysids, shrimps were next, and polychaetes and clams were of minor relative importance. The category "other" mainly consisted of unidentified food items, but included a few pea crabs (family Pinnotheridae), gastropods, brittle stars, and isopods.

One species of mysid, *Neomysis kadiakensis*, was identified, and two genera of shrimps, *Pandalus* and *Crago*. By far the commonest fish was *Clupea pallasi*, other species being the two zoarcids *Lycodes brevipes* and *Lycodopsis pacifica*, and *Spirinchus thaleichthys*, and *Lumpenus sagitta*. *Yoldia* sp. was the commonest clam, *Nucula tenuis* being next; *Serripes groenlandicus* and *Pandora filosa* were identified from a few stomachs.

Food habits varied with size of sole and time of year (Fig. 1). Mysids were important contributors to the diet in all size groups, although pri-

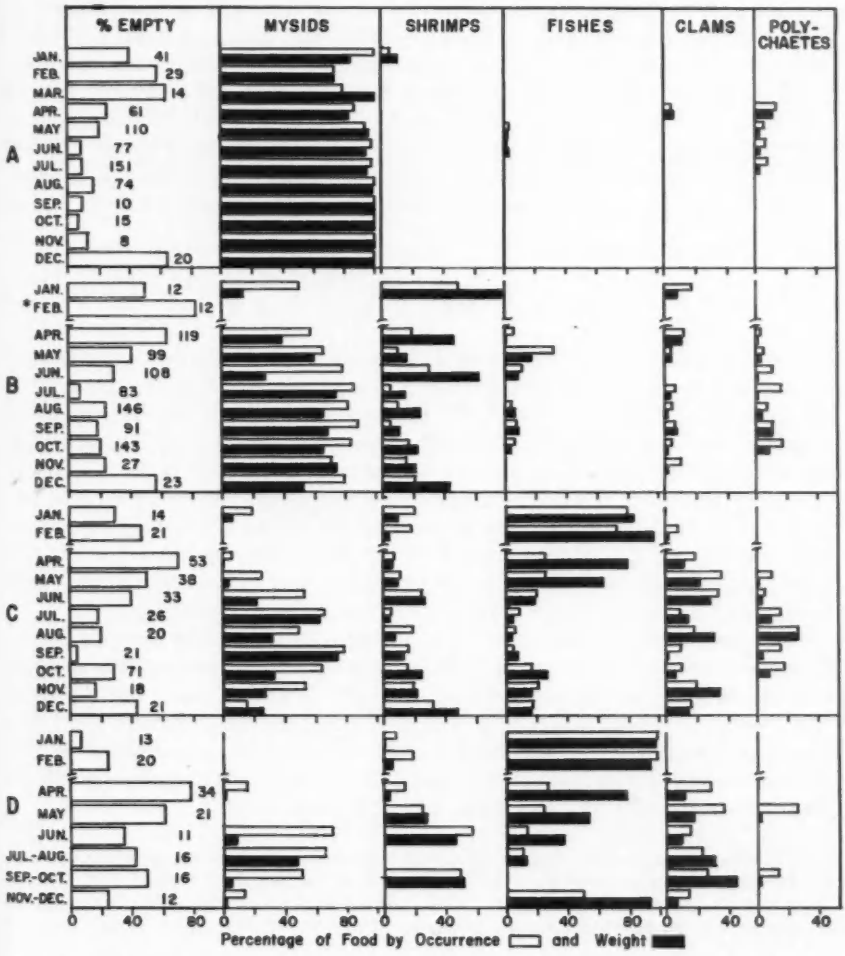

FIG. 1. Relative importance of major food categories by size group of sole and month. The number of stomachs examined in the various months are indicated under "% Empty." A, 40–179 mm total length (TL); B, 180–259 mm TL (*, only two stomachs with food, 1 mysid and 1 fish); C, 260–319 mm TL; D, 320–440 mm TL.

marily only in the summer and fall in the larger sole. Shrimps were important in the diet of sole 180 mm and larger. Fishes in particular, and clams less so, were important in the diet of sole 260 mm and larger; fishes were the major food item of these sole in the winter and spring. Polychaetes were of some importance in the diet of medium-size sole in the summer and early fall.

The percentage of empty stomachs also varied with size of sole and time of year (Fig. 1). The percentage empty in the 40- to 179-mm group was largest from December to March, averaging 54; in the 180- to 259-mm group, from December to May, averaging 55; in the 260- to 319-mm group, from December to June, averaging 49; and in the 320- to 440-mm group in April and May, averaging 73, although it was also high from July to October, averaging 47. A diminution of feeding (as measured by percentage of empty stomachs) was associated with the spawning period of the sole, which was observed in the field to peak in April. Only the smallest group of mainly immature sole (40–179 mm) did not show a high percentage of empty stomachs during April.

Bottom temperature data (C) obtained for each collection month was as follows:

Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
7.4	5.9	7.1	8.2	8.6	8.9	10.5	10.9	11.8	10.7	10.1	8.3

There was some indication of an association between the percentage of empty stomachs (Fig. 1) and water temperature. The percentage of empty stomachs in small, immature fish (40–179 mm) dropped substantially during the same time the water temperature increased from 7.1 (March) to 8.2 (April). All sole except the largest group (320–440 mm) showed an increase in the percentage of empty stomachs when the temperature dropped from 10.1 (November) to 8.3 (December); the largest group had its lowest percentage of empty stomachs during the months of the lowest water temperatures. During this period large schools of spawning herring were observed in East Sound, and these were found to be the major food item of the large flathead sole.

Moiseev (1953) indicated the genus *Hippoglossoides* to be a characteristic flounder of mixed feeding habits. The findings in this study essentially support this characterization of the flathead sole (*H. elassodon*) since both benthic and nektonic food organisms were found in the stomachs, although the latter clearly predominated. Moiseev further characterizes this genus of fish as having reduced feeding during the colder months of the year; however, in the present study the larger fish did not show any indication of this.

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